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ORIGINAL ARTICLES

THE INCIDENCE OF AIRBORNE HORMODENDRUM AND ALTERNARIA SPORES AS DETERMINED BY A VOLUMETRIC SAMPLER

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CERTAIN airborne spores of fungi have gradually been accepted as an important cause of allergic reactions. The atmospheric incidence of airborne spores has been determined almost entirely by the use of two standard sampling methods—(1) the plate method, which is considered best for identification, and (2) the slide method, which is used for those spores that can be identified microscopically and to determine seasonal fluctuations.

A few volumetric samplers have been used in an attempt to measure more accurately the incidence of certain airborne spores. Durham[1] employed a volumetric device devised by Hawes[2] and obtained simultaneous gravity slide counts. In a comparison of the number of alternaria spores collected by these two sampling methods, he found that there was a marked difference in the day-to-day ratios in the number of spores collected and suggested that gravity slide counts must be regarded as being much more inaccurate than many workers have assumed.

Alvarez and Castro,[3] recognizing the limitations of the culture plate method and attempting to determine quantitatively the spore count of air, designed a four-sided wooden box on the bottom of which was placed an exposed culture plate. Preliminary experiments indicated that the spores in this closed box would settle out of the air in one hour. After exposure, the plate was incubated and the colonies were counted. Knowing the dimensions of the box and the surface area of the exposed plate, Alvarez and Castro reported these counts as colonies per cubic meters of air. They found in Havana that the greatest concentration of fungi is at night.

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Hirst,[4] in England, used a power-operated suction trap by which spores are impacted onto the sticky surface of a slowly moving slide, a sampling method similar to that employed in the Hawes impinger. During a fourteen-day period in June Hirst found a marked diurnal variation of pollen and cladosporium (hormodendrum). Maximal concentrations of both were usually reached in the afternoon, with the lowest concentration occurring shortly after midnight. Although the incidence of alternaria was much lower than that of hormodendrum, Hirst reports a similar diurnal variation for alternaria.

STATEMENT OF PROBLEM

This is a volumetric concentration measurement study of airborne alternaria and hormodendrum spores. It was hoped that further study of these two spores with a volumetric sampler would give a clearer picture as to their actual incidence and their diurnal and hourly variation, if any, and that a comparison with the standard gravity slide method would help in the interpretation of these daily counts.

PROCEDURE

The volumetric sampler used was constructed, with minor alterations, according to instructions received from Stenburg and Hall.[5] The principle involved in sampling is impingement of particles in a given volume of air onto the adhesive side of moving cellulose tape and the covering of this collecting surface during sampling with a facing piece of adhesive tape. Movement of the tape during sampling spreads out the collected spores over a sufficient area to make counting and identification accurate although time-consuming.

Both the volumetric sampler and the standard gravity sampler were located on the topmost ledge of a tower above the roof of the Medical Laboratories Building in Iowa City, Iowa. The standard gravity sampler was located at the same height and just a few feet distant from the volumetric sampler, so that a comparison was possible in the incidence of spores collected by both samplers. The results reported here are based on readings taken during the month of August, 1952, and during a twelve-day period in August, 1956.

The sampler was necessarily stopped for a few minutes at 9 A.M. each day in order that the portion of the tape representing the previous twenty-four hours of sampling might be removed. This portion of the tape was divided into twenty-four equal parts, with each representing one hour of sampling time and approximately one cubic yard of air. All hormodendrum and alternaria spores were identified and counted under high-power magnification and in terms of the volumetric sampler expressed as spores per cubic yard of air. During the counting of all hormodendrum spores on both the slides and the tapes, a recording was made as to whether the spore occurred singly or in clumps (masses or aggregates) and, if in clumps, the number of spores per aggregate.

The efficiency of this volumetric sampler was found in a previous study,[6] under certain experimental conditions, to be 76 per cent for commercially collected ragweed pollen (*Ambrosia trifida*). The efficiency is not known for

spores, but it must be less than for ragweed pollen because of their smaller size. Similarly, the sampling efficiency is less for hormodendrum than for alternaria because of their smaller size. Although the sampling efficiency of this instrument is low for these two allergens, the volumetric readings do give pertinent information as to the exposure of the patient to these two spores in that the sampling rate approximates the volume of air inspired by an adult during normal activity.

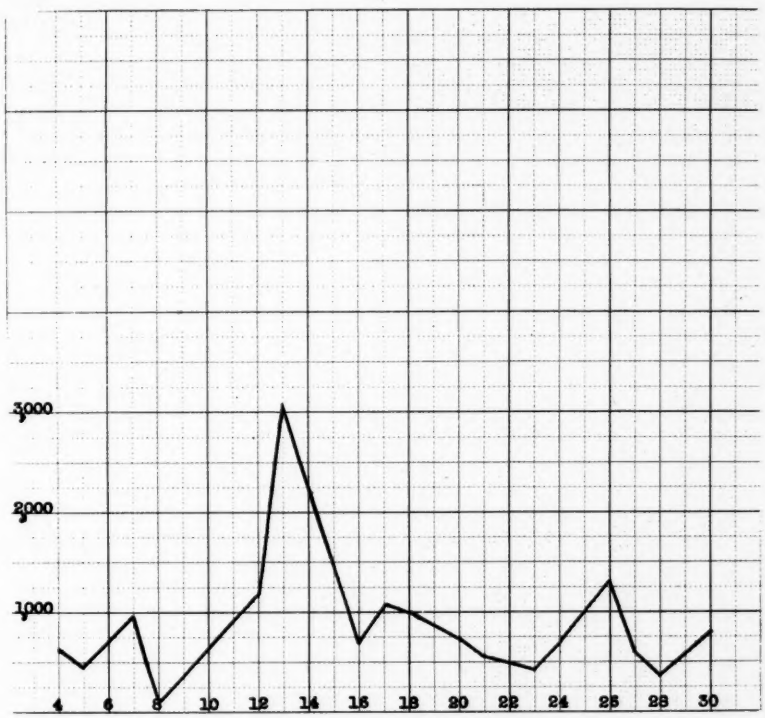

Fig. 1.—Daily alternaria spore count, August, 1952, Iowa City, Iowa.

DISCUSSION AND RESULTS

Daily Incidence of Airborne Alternaria and Hormodendrum Spores.—Figs. 1 and 2 indicate the marked daily variation in the aerial concentration of these two spores. Each daily reading represents approximately 24 cubic yards of sampled air. Although hormodendrum always had the greatest daily incidence, the difference in daily totals fluctuated markedly from day to day. Eighty-eight times as many hormodendrum spores as alternaria spores were collected on August 8, and the ratio was 50 to 1 on August 4, while on August 23 the hormodendrum count only slightly exceeded that of alternaria. These sudden daily changes and these changing ratios indicate how rapidly the spore content of the air can undergo alteration. Certain factors that might be expected to influence this count, such as atmospheric temperature, soil temperature, sunshine, turbulence, and humidity, do not have the same effect on both spores if

they are important in influencing atmospheric spore counts. The peak incidence of both spores was reached on August 13. There were no known unusual weather changes or meteorological factors that can be used to explain this high incidence of both spores on this particular day. Readings taken during the month of August, 1956, show the same daily changes and similar changing ratios in the number of spores collected.

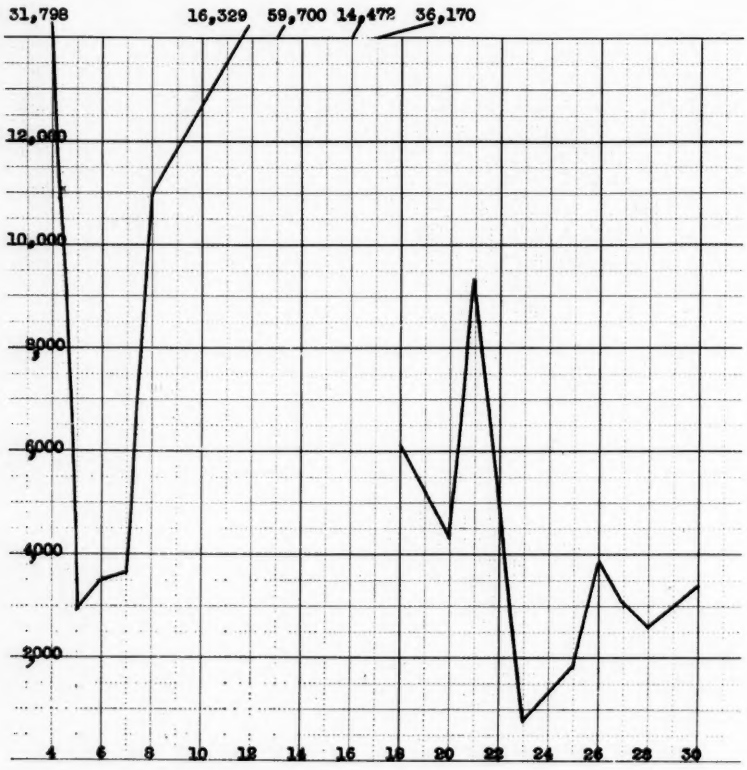

Fig. 2.—Daily hormodendrum spore count, August, 1952, Iowa City, Iowa.

The more marked daily changes in the occurrence of hormodendrum spores were not due to a change in the number of spores per clump. On some days the total hormodendrum count was high and the number of spores per clump was small, while on other days the total count was low and the number of spores per clump was twice that of days with a high incidence.

A comparison of day-and-night total counts shows that both spores had their highest incidence during the day (6 A.M. to 6 P.M.). For the month of August the alternaria and hormodendrum counts were approximately 23 per cent higher during the daylight hours. In three instances the number of alternaria spores collected during the night exceeded that of the daytime readings, and on one of these same days the hormodendrum count was higher at night.

On eight days during the month of August there was a fall in incidence of hormodendrum spores during the night and in one instance this amounted to a 90 per cent reduction. In each instance there was an associated fall in the

alternaria counts, although the decrease was less than that for hormodendrum. This decrease was not associated with rainfall, as even a small shower destroyed the adhesive qualities of the sampling tape and if a rain occurred during a twenty-four-hour period the sampling tape was discarded. The number of hormodendrum spores per clump was usually slightly greater during the day as compared to night readings, but a change in clumping does not account for this decrease in hormodendrum spores at night on these eight occasions.

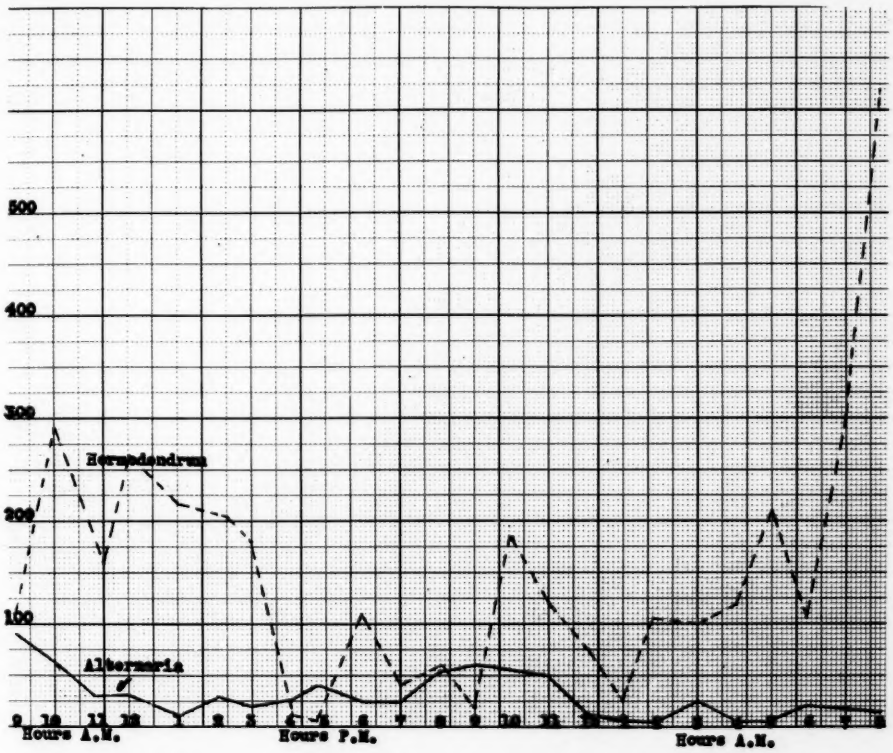

Fig. 3.—Hourly variation of airborne hormodendrum and alternaria spores. Gravity slide count per square centimeter slide area: hormodendrum, 5; alternaria, 14. Aug. 7, 1952, Iowa City, Iowa.

Hourly Incidence of Airborne Alternaria and Hormodendrum Spores.—Figs. 3, 4, and 5 show the marked hourly variation in the number of these two airborne spores. These three days are representative of the two sampling periods in that every day was characterized by abrupt hourly changes in the number of spores sampled. A comparison on an hourly basis indicates little relationship in the peak incidence of these two spores. On four days during the month of August, however, the maximum count of each was reached at the same hour of the day, and in each instance this occurred during the morning hours.

The peak incidence of alternaria spores was reached on every day during the daylight hours and in all but three days during the morning hours. Hormodendrum spores showed a greater variation in that on two occasions during the month the greatest hourly concentration occurred at night, although on all other days the peak incidence was also during the morning hours.

The total number of spores collected during August was highest during the period from 6 A.M. to noon (hormodendrum 54 per cent, alternaria 35 per cent). The lowest incidence during the month was from 6 P.M. to midnight (hormodendrum 11 per cent, alternaria 18 per cent).

These sudden hourly changes indicate that there is no homogenous mixture of spores in a given volume of air. It appears more likely, as suggested by Gregory,[7] that spore clouds are formed. These results show that in some instances these clouds are made up of large numbers of both hormodendrum and

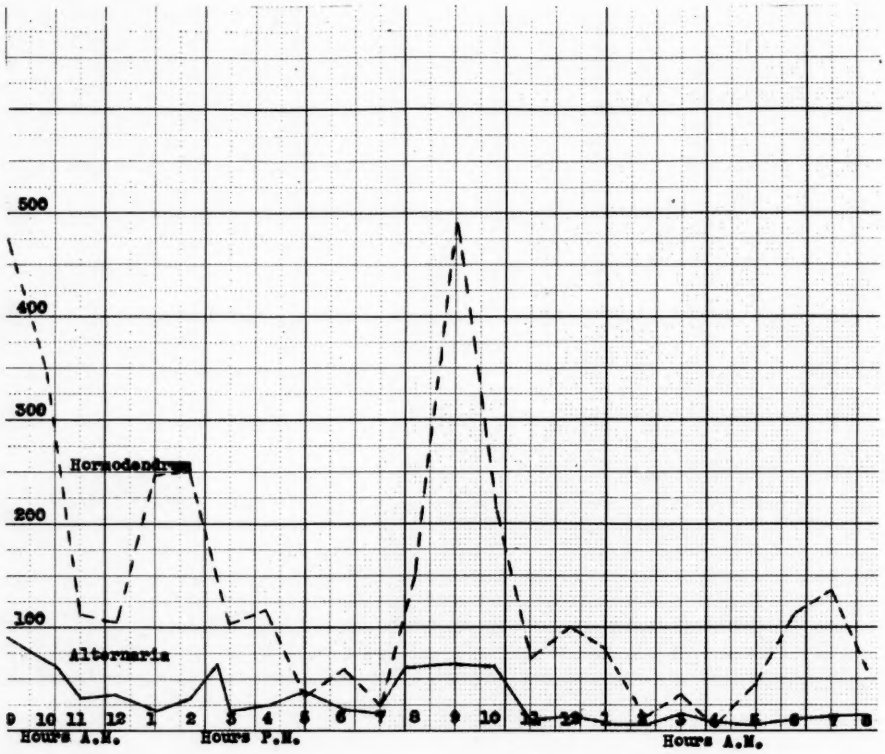

Fig. 4.—Hourly variation of airborne hormodendrum and alternaria spores. Gravity slide count per square centimeter slide area: hormodendrum, 6; alternaria, 22. Aug. 6, 1952, Iowa City, Iowa.

alternaria spores, as when the maximum hourly incidence is the same for both spores. Usually, however, these clouds are composed of alternaria or hormodendrum spores, as is indicated when the peak incidence of both spores is not the same and in the many instances in which there is little agreement in the hourly counts.

These spore clouds of both spores occurred more commonly during the morning hours, but they do take place at any time of the day or night, as is shown by the sudden increase of spore counts that may occur at any time. These abrupt hourly changes emphasize the suddenness of air spore changes and indicate how difficult it would be, because of a lack of uniformity in these counts, to try to explain these changes in terms of weather or meteorological conditions.

It has been previously pointed out[8] that when volumetric readings were taken at twelve-minute intervals there was little agreement between these readings in terms of the number of hormodendrum and alternaria spores collected. These readings indicate that volumetric readings of one hour in length, even if taken at the same time each day, cannot be used to indicate the daily incidence of these two spores.

Volumeric Incidence of Alternaria and Hormodendrum Spores as Compared With Gravity Slide Counts.—Slide counts and plate counts are in rather poor agreement[9] for both alternaria and hormodendrum spores. Because of the much longer exposure time of slides by the gravity method as compared to culture plates that are exposed for twelve minutes, one might suppose that slide counts would more nearly reflect the incidence of these two airborne spores.

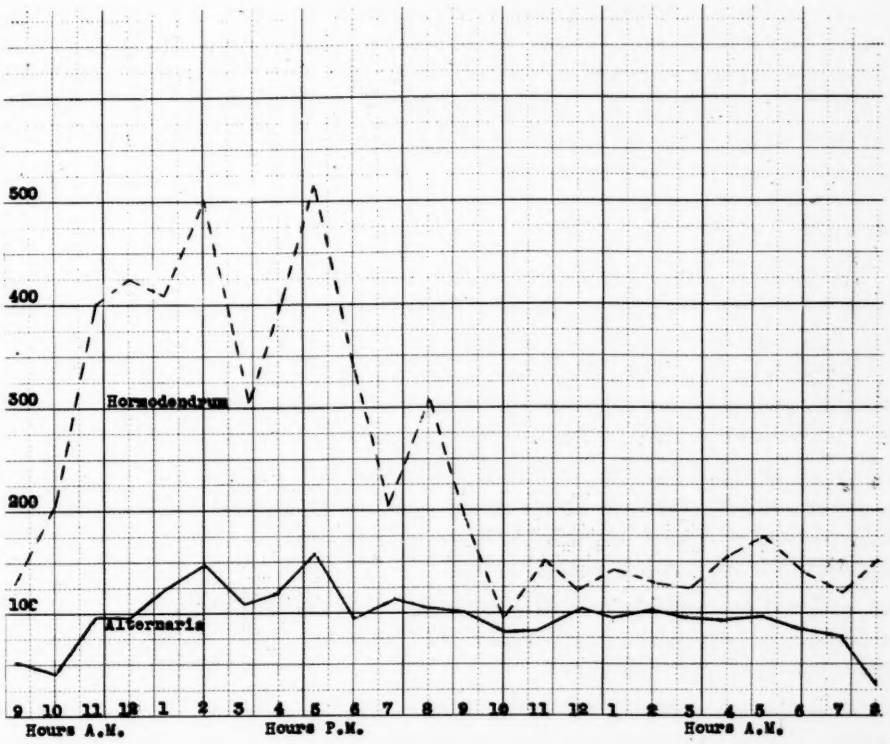

Fig. 5.—Hourly variation of airborne hormodendrum and alternaria spores. Gravity slide count per square centimeter slide area: hormodendrum, 26; alternaria, 8. Aug. 9, 1956, Iowa City, Iowa.

In this study a direct comparison of the incidence of these two spores in terms of a volumetric sampler and the gravity slide method was possible during the two sampling periods of 1952 and 1956. Figs. 3, 4, and 5 show the hourly slide counts on three representative days during these sampling periods. These slide counts do not suggest the high atmospheric concentration of these spores when a comparison is made with the hourly volumetric incidence, and they are still less suggestive as to the occurrence of these spores when a comparison

is made with the daily volumetric incidence. August 6 (Fig. 1) alternaria, with a gravity slide count of 22, represents 766 alternaria spores collected volumetrically on this day. Similarly, the gravity slide count of 22 represents 3,416 hormodendrum spores collected volumetrically (Fig. 2). On August 7, another day with a comparatively low volumetric incidence of these two spores—990 for alternaria and 3,681 for hormodendrum—the gravity slide counts were alternaria 8 and hormodendrum 26. If we take the peak incidence during the month of August, in terms of both spores, which was reached on August 13, the gravity slide counts were alternaria 19 and hormodendrum 10. Yet on this day, according to the volumetric sampler, the individual inspired during the course of twenty-four hours some 3,000 alternaria and 59,700 hormodendrum spores.

Durham[1] has pointed out the marked daily variation when ratios are computed for the gravity slide and the volumetric recovery of alternaria spores. A similar variation was found in this study, in that the daily ratios for alternaria varied from 1 to 30 to 1 to 386. A still greater fluctuation occurred in ratios for hormodendrum spores, 1 to 369 to 1 to 6,359. These changing daily ratios indicate that about the only interpretation that can be placed on gravity slide counts in terms of these two spores is that this count indicates that the two spores were airborne on a given day.

SUMMARY

Air samples for hormodendrum and alternaria spores were taken simultaneously by the use of a volumetric sampler and a standard gravity sampler. Both samplers were located on the topmost ledge of a tower above the roof of the Medical Laboratories Building in Iowa City, Iowa. The results here reported are based on readings taken during the month of August, 1952, and during a twelve-day period in August, 1956.

1. Volumetric readings indicated that there is a marked daily variation in the aerial concentration of these two spores and, although the hormodendrum count always exceeded the alternaria count, computed ratios indicated little relationship in their daily concentration.

2. The total number of both spores collected volumetrically during August was highest during the period from 6 A.M. to noon, with the lowest total incidence from 6 P.M. to midnight.

3. Although usually the peak hourly concentration of both spores was reached during the morning hours, a sudden hourly decrease or increase did occur at any time.

4. A comparison of volumetric counts with the gravity slide counts shows that the only interpretation that can be placed on slide counts is that the two spores were airborne on a given day.

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STUDIES IN ALLERGY

II. Serum Serotonin Levels in Nonallergic, Pretreatment, and Posttreatment Allergic Human Beings and in Normal and Sensitized Guinea Pigs

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CONSIDERABLE controversy exists in the present-day biochemical and allergic literature concerning the role of serotonin (5-hydroxytryptamine) in allergic shock reaction and anaphylaxis. The presence of high serotonin levels in the lungs of the mouse, rat, and rabbit has been correlated with antihistamine-resistant anaphylactic shock in these animals.[1] The demonstration of increasing serotonin sensitivity following in vivo anaphylactic shock in the sensitized virgin guinea pig uterus[2] suggested that serotonin may play a role in some allergic and anaphylactic manifestations and that a clue might be found in the serum serotonin levels of patients with allergic sensitivity.

The present investigation was set up to study the serum serotonin levels of normal and sensitized guinea pigs. The serum serotonin levels of a group of normal nonallergic patients, as well as a group of allergic patients who entered the Allergy Inpatient and Outpatient Services of the Omaha Veterans Administration Hospital, were studied before and after treatment with water-soluble antigens.

METHOD

Serum serotonin levels were determined spectrofluorometrically. The method[9] used was a modification of the procedure described by Waalkes and associates.[3] In the present study, 2.5 ml. of double-distilled water (having minimal fluorescence) was added to 1 ml. of serum. Then 1 ml. of 10 per cent zinc sulfate followed by 0.5 ml. 1 N sodium hydroxide was added to deproteinize the serum. After centrifugation, 1 ml. of the clear supernatant was combined with 2 ml. of 9 N sulfuric acid. The fluorescence of the acidified sample was then determined in a Farrand spectrofluorometer (activation, 300 mμ; fluorescence, 550 mμ). By addition of 0.002 micromoles of serotonin to a duplicate serum sample, we obtained an internal sample which was used for purposes of calculation.

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The Animal Data.—The initial serum serotonin blood levels were obtained on forty-six female guinea pigs, each weighing 300 grams. Subsequently, eighteen of these animals were injected subcutaneously with alum-precipitated antigens, egg albumin, feathers, ragweed, and house dust. The serum serotonin levels of these animals were then studied monthly until the animals were sacrificed by intracardiac injection of antigen and the uterus was tested with water-soluble antigen in the Schultz-Dale apparatus. These animals were killed by a blow on the head, and the uteri were tested in the Schultz-Dale apparatus against water-soluble antigen.

The experimental group contained those animals that were anaphylactically sensitive to antigens (egg albumin, feathers, house dust, or ragweed) as evidenced by gross anaphylactic death and by contraction in the Schultz-Dale bath containing antigen.

The Human Data.—Forty-nine normal male medical students, physicians, laboratory technicians, and other hospital employees voluntarily supplied blood for normal serotonin levels. These persons had no allergic histories and were under no undue stress at the time the blood was taken.

Thirty-one allergic patients who suffered from a variety of allergic manifestations, including vernal conjunctivitis, allergic rhinitis, and seasonal bronchial asthma, were studied for serum serotonin levels. Thirty of these were placed under therapy with appropriate aqueous extracts of antigens, dust, ragweed, hemp, red top and, in several cases, tobacco and studied for serotonin serum levels after at least eight weeks of therapy with antigen doses adjusted to individual tolerance.

RESULTS

The mean serum serotonin level of the female guinea pigs in the normal group was found to be 0.285 mcg. ± 0.110 mcg. per cubic centimeter (Table I).

TABLE I. SERUM SEROTONIN LEVELS IN NORMAL GUINEA PIGS AND GUINEA PIGS SENSITIZED TO ANTIGENS

	(A) NORMAL GUINEA PIGS	(B) SENSITIZED GUINEA PIGS
Number	46	13
Mean serotonin level	0.285 mcg./c.c.	1. Initial 0.249 mcg./c.c. 2. Postsensitized 0.254 mcg./c.c.
Range	0.155-0.704	1. 0.176-0.303 2. 0.134-0.475
Standard deviation	0.110	1. 0.085 2. 0.107
Relationship of "t" test and significance	A to B_1 A to B_2 B_1 to B_2	1.09 P >0.05% 0.886 P >0.05% 0.132 P >0.05%

This is higher than that seen in the human subjects. Thirteen guinea pigs which were anaphylactically sensitized showed no statistically significant difference in serum serotonin level by "t" test, as noted in Table I, when tested initially and retested at a two-month interval after sensitization. The mean serotonin levels for the serum of this group of guinea pigs were 0.249 ± 0.085 mcg. per cubic centimeter before sensitization and 0.254 ± 0.107 mcg. per cubic centimeter after sensitization.

The results of the human serotonin levels are shown in Table II. The normal level for serum serotonin is 0.1527 ± 0.0668 mcg. per cubic centimeter and, when converted by hematocrit values for whole blood, agrees with levels found in the literature[8] for the normal control subject.

TABLE II. SERUM SEROTONIN LEVELS IN NORMAL PERSONS, ALLERGIC PATIENTS, AND POSTTREATMENT ALLERGIES

	(A) NORMAL PERSONS	(B) PRETREATMENT ALLERGIC PATIENTS	(C) POSTTREATMENT ALLERGIC PATIENTS
Number	49	31	30
Mean serotonin serum level range	0.1527 mcg./c.c. 0.075-0.227	0.2047 mcg./c.c. 0.065-0.932	0.1972 mcg./c.c. 0.081-0.458
Standard deviation	0.0668	0.1566	0.0789
Relationship of "t" test and significance	A to B B to C A to C	2.05 P $<$0.05	 0.235 $>$0.05 2.696 $<$0.05

The serum serotonin level in pretreatment allergic persons is 0.2047 ± 0.1566 mcg. per cubic centimeter. This was considerably higher than the level found in normal persons. Statistical analysis for significance, by use of the "t" test, demonstrates the significance of this difference to be greater than at the 0.05 level.

Following allergic hyposensitization therapy with aqueous antigen extracts, the serum serotonin level remains high, the mean for this group being 0.1972 ± 0.0789 mcg. per cubic centimeter. The statistical difference between the mean of this group and that of the pretreatment allergic group is below the level of 0.05 significance, but it is above the 0.05 level of significance when compared with normal nonallergic human beings.

DISCUSSION

The role of 5-hydroxytryptamine (serotonin) in the body is complex because of its manifold properties and functions. The compound does not exist in whole blood or plasma, but it is found in serum as the result of platelet disintegration and in the brain, lungs, and small bowel. Humphrey and Jacques[4] demonstrated its presence in rabbit serum in high concentrations following anaphylactic shock and suggested that the platelet's rapid disintegration during anaphylaxis was the etiological factor and the source of serotonin. Comroe and his associates[5] studied the reflex and direct cardiopulmonary effects of serotonin in cats and found that minute doses of the order of 0.5 mcg. per kilogram of

body weight produced profound reflex cardiopulmonary changes, including bradycardia, hypopnea, and apnea, with bronchoconstriction and pulmonary vasoconstriction. These effects are mediated by serotonin-sensitive afferent fibers of the vagus nerve which are supplied by blood from the pulmonary circulation and ascending aorta.[5] Herxheimer,[6] working with aerosolized serotonin in the tracheobroncheal tree of the guinea pig, was able to produce a shock syndrome in the guinea pig similar to that caused by anaphylaxis, histamine, and acetylcholine. Guinea pigs sensitized to egg albumin and then desensitized by multiple antigenic exposure developed increasing tolerance to serotonin. Weissbach and co-workers[1] showed the presence of significant concentrations of serotonin of the order of 2.0 mcg. per gram in the lung tissue of animals sensitive to serotonin, such as the mouse, rat, and rabbit, and much smaller amounts in the guinea pig which is (0.2 mcg. per gram) a relatively nonsensitive species. It is interesting to note that the levels of monoamine oxidase (an enzyme thought to be a catalytic oxidizer of serotonin) were significantly higher in the guinea pig.[3] These authors suggest that in those species in which serotonin is found in the lungs in significant quantities both histamine and serotonin should be considered as synergistic factors in allergic and anaphylactic reactions.

The higher level of serotonin which we have noted in the sera of persons with allergic sensitivity may be a response to allergic stress induced by contact of a sensitive person with an antigen or multiple antigen. Perhaps the platelets are sensitized as are the rest of the body tissues and respond by releasing excess serotonin, as suggested by Humphrey and Jaques.[4]

Fink and Gardner[7] failed to demonstrate inhibition of anaphylaxis in guinea pig uteri and ileum in the Schultz-Dale apparatus by reserpine, yohimbine, and lysergic acid, and they postulate that serotonin plays no role in in vitro anaphylaxis. We agree with this concept as it applies to the guinea pig uterus in the Schultz-Dale bath. Our studies[2] and those of Fink and Gardner[7] have shown that the guinea pig uterus and ileum are poor test organs for serotonin because of the low levels of positive responses (31 per cent of normal uteri and 40 per cent of sensitized uteri) in our series. We have also noted that a statistically significant increase in serotonin sensitivity occurs in this organ with in vivo anaphylaxis. We have postulated that the antigen-antibody—cellular membrane reaction in some unknown way sensitizes the cell to respond maximally to a serotonin level which formerly produced little or no response.[2]

Hyposensitization therapy appears to have no effect upon serotonin levels of the serum of posttreatment allergic patients. This was to be expected, since repeated exposure to antigen would tend to cause repeated stimulus for releasing of serotonin. In guinea pigs, however, there is no change in serotonin blood level as sensitization proceeds. This again demonstrates another essential difference between human atopy and anaphylaxis in animals.

SUMMARY

The sera of normal guinea pigs, sensitized guinea pigs, normal human beings, pretreatment allergic human beings, and posttreatment allergic human

beings have been examined for serotonin levels. Significant differences exist in allergic human beings in the direction of increased serotonin levels. These differences are not changed by allergic hyposensitization with appropriate antigens. In the guinea pig serum levels of serotonin do not change with sensitization. The meaning of these findings is discussed with reference to recent work on the role of serotonin, histamine, and antigen-antibody complexes in allergy and anaphylaxis.

We wish to express our gratitude to Dr. Morris Brodkey, who is in charge of the Outpatient Allergy Section and who furnished us with sera, time, and patient counsel.

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STUDIES ON THE ALLERGENS OF RAGWEED POLLEN

A Review

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INTRODUCTION

THE first recorded observation of the condition referred to today as hay fever was made in 1565 by Botallus,[1] who noted that some persons began to sneeze when exposed to certain flowers.

In 1819 Bostock[2] described the symptoms of the disease, and it was subsequently known as "Bostock's catarrh." Evidence that this catarrh might be due to pollen was presented in 1831 by Elliotson,[3] who observed that the period of worst symptoms coincided with the maturing of grasses (pollination) in the haying season. Because of its apparent relationship with the season of the year, the disease became known as hay fever. The works of Gordon,[4] Phoebus,[5] Salter,[6] and Wyman[7] all implicated pollen as the causative agent. Blackley,[8] in 1865, performed the first diagnostic skin test for hay fever and also showed that the pollen of grasses was capable of inducing conditions identical to hay fever in allergic persons. Dunbar,[9] who accepted the view that the pollen of grasses was responsible for hay fever, repeated the main experiments of Blackley and established beyond doubt that the pollen was the causative agent. In 1910, Meltzer[10] showed that a patient suffering from hay fever was sensitive to a constituent of the pollen.

Many attempts have been made during the past four decades to isolate and characterize the allergen or allergens in ragweed pollen, but almost all have met with little success. This is not surprising in view of the complexity of the structure and chemical composition in ragweed pollen. On the American continent there are two main varieties of ragweed. Both are annual herbs. Short or dwarf ragweed *(Ambrosia artemisiaefolia)* does not exceed 4 feet in length, and giant or tall ragweed *(Ambrosia trifida)* reaches a length of about 15 feet. The pollen grains of both ragweeds are similar in structure. They are almost spherical, 16 to 20 microns in diameter, and are bright yellow in color. The outer cell wall forms a very conspicuous portion of the cell (about 65 per cent of the weight of the pollen) and is composed of a hard

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and seemingly inert material. The inner cavity of the pollen consists of cytoplasm, cytoplasmic granules, and two nuclei, all of which are separated from the outer cell wall by a thin membrane composed chiefly of cellulose. The pollen grains are endowed with the potentialities of a living cell and therefore contain all the chemical constituents necessary for reproduction, such as enzymes, nucleoproteins, carbohydrates, proteins, lipids, peptides, and pigments.

ALLERGENIC IDENTITY OF POLLENS OF GIANT AND SHORT RAGWEED

Since many investigators used pollen of either giant or short ragweed and some made no mention of the type of pollen used, the allergenic relationship between the two pollens must be pointed out. Spain and Hopkins,[11] using the method of cross-neutralization of sites sensitized with sera of persons allergic to both pollens, failed to find any specific differences in the allergens of the two pollens. Similar results were obtained by Stull and associates,[12] who also showed that the extract of short ragweed pollen was as effective as that of giant ragweed pollen in the treatment of hay fever. Brown[13] reported that many patients suffering from hay fever were equally sensitive to extracts of giant and short ragweed pollen. After such allergic persons were treated with the extracts of one of the pollens, the injection of an equivalent or larger quantity of the other pollen extract into any of these persons was not followed by a constitutional reaction, which would have occurred had the allergens of the two pollens not been identical. Coca and Grove,[14] employing the method of passive transfer, found that sensitized sites after neutralization to an extract of low ragweed pollen did not react to an extract of giant ragweed pollen and vice versa. The sera used in their experiments were from patients allergic to both types of ragweed pollen.

Thus, it would appear that the allergens of giant and short ragweed pollen are identical.

ANTIGENICITY OF RAGWEED POLLEN

Although precipitating antibodies to ragweed pollen extracts have never been found in sera of allergic persons, ragweed pollen has been found to be antigenic in both rabbits and guinea pigs, precipitating antibodies consistently being formed by both of these animals.[15-30] A large number of investigations have demonstrated that ragweed pollen contains several antigens.[18, 20-24, 29] For example, Wodehouse[22, 23] has demonstrated the existence of at least seven antigens, and more recently Cebra[29] has been able to identify as many as ten antigenic components in ragweed pollen extracts by gel diffusion methods.

Harrison and Armstrong[32] and Walzer and Grove[33] concluded that the antigens of giant and short ragweed pollens were identical with respect to antibodies induced in guinea pigs. They found that the uterus of a guinea pig sensitized to an extract of giant ragweed pollen was sensitive to both pollen extracts and that after desensitization of the uterus with either pollen extract it was insensitive to both. In order to correlate the antigenicity of

ragweed pollen with its allergenicity, however, it must be ascertained that the antigens in ragweed pollen which elicit formation of precipitating antibodies in laboratory animals are identical to the allergens responsible for hay fever in man.

Black[34] showed that ragweed pollen extracts digested with trypsin were no longer able to act as antigens in complement-fixation tests (using human allergic serum), although they were allergenically as active as before digestion. Armstrong and Harrison[35] could find no relationship between the complement-binding capacity of the pollen extract (using rabbit antisera) and its ability to produce skin reactions in allergic persons. Mosko and colleagues,[15] Roth and Nelson,[16] and Hecht and co-workers[17] all succeeded in separating extracts of the pollen into fractions which were either allergenically active or able to give positive precipitin tests with rabbit antiragweed pollen sera. The available evidence would, therefore, suggest that the allergens and antigens in ragweed pollen are distinct entities.

PRELIMINARY STUDIES OF THE CHEMICAL COMPOSITION OF RAGWEED POLLEN

In 1914 Heyl commenced a series of analyses on ragweed pollen. In his initial communication,[36] he reported the composition of the pollen to be as follows: moisture, 5.3 per cent; phosphorus, 0.37 per cent; alcohol-soluble material, 42.9 per cent; crude fiber (mostly cellulose), 12.2 per cent; pentosans, 7.3 per cent; dextrin, 2.1 per cent; ash, 5.4 per cent; and protein, 24.4 per cent. He assumed that all the nitrogen-containing material was protein. The ether-soluble fraction, obtained on defatting the ragweed pollen, amounted to about 12.5 per cent of the dry weight of the pollen. In a subsequent study,[37] he showed that the water-soluble extract of the pollen, obtained after the pollen was defatted with ether and extracted with 95 per cent alcohol, contained peptones, adenine, guanosine, histidine, arginine, lysine, and several other not well-characterized constituents. In addition to these components, his extract contained two different proteins—(1) an albumin, which coagulated at 45 to 50° C. in the presence of small amounts of acetic acid, and (2) a proteose, which could not be coagulated by heat, but which could be precipitated by saturating the solution with ammonium sulfate. Extraction of the pollen with dilute alkali after its extraction with water yielded a glutelin fraction which contained histidine, whereas the albumin and proteose fractions did not.[38] All three protein fractions were allergenically active in ragweed-sensitive persons. Heyl also demonstrated[39] that the yellow pigments in ragweed pollen were flavonols, which are constituents of many flowers. Two of the pigments that he isolated were isoquercitin (a quercitin-glucoside), and isorhamnetin (a monomethyl ether of quercitin). In a study of the ether-soluble fraction of the pollen,[40] he demonstrated the presence of formic, acetic, valeric, lauric, oleic, palmitic, and myristic acids. Heyl concluded that none of the substances which he showed to be present in ragweed (he identified twenty-seven substances) appeared to "represent any chemical specialization of the cell." He did not attempt to correlate allergenic activity with all the fractions that he isolated.

A similar but less comprehensive study on the chemical composition of ragweed pollen was carried out at the same time by Koessler.[41] This investigator determined the total nitrogen of the whole pollen and its distribution among the different proteins isolated, and he also showed that ragweed pollen did not contain starch.

These studies are important in that they demonstrate the complexity of ragweed pollen and of the aqueous extract of the pollen. However, they do not offer any aid in elucidating the nature of the allergen or allergens present in the pollen.

EVIDENCE IN SUPPORT OF THE PROTEIN NATURE OF THE ALLERGEN(S) IN RAGWEED POLLEN

In 1906, Wolff-Eisener[42] discussed the possibility that hay fever might be the result of sensitivity to a protein component of the pollen and that the condition might be similar to anaphylactic sensitivity, which can be induced in animals. In 1911, this hypothesis was further supported by Noon,[43] who assumed that hay fever was a type of anaphylactic sensitivity. Noon claimed to have succeeded in desensitizing patients suffering from hay fever by the injection of water-soluble extracts of the pollen. Only proteins were then thought capable of inducing the formation of antibodies, and consequently desensitization was interpreted as being due to the formation of neutralizing antibodies by the protein constituents of the injected pollen extracts. In confirmation of this belief, Koessler[44] stated in 1914 that pollen preparations in which no protein could be detected were devoid of allergenic activity.

Bernton and colleagues,[45] Rappaport and Johnson,[46] and Moore and associates[47] isolated protein fractions from ragweed pollen extracts by precipitation with either ammonium sulfate or alcohol. All the fractions were found to be active by skin test in allergic persons, although variations in the sensitivity of the patients to the protein fractions were noted.[45, 46]

Spain and Newell[48] demonstrated that they could completely remove the nonprotein nitrogen from ragweed pollen extracts by ultrafiltration without appreciably diminishing the activity.

Unger and associates[49] prepared a protein and a carbohydrate fraction from an aqueous extract of the pollen. They found that passively sensitized sites neutralized to the protein failed to react to the carbohydrate, whereas sites neutralized to the carbohydrate were still capable of reacting to the protein, and concluded that the allergen was a protein.

Stull and co-workers have been the most active proponents of the theory that the allergen was a protein. In 1931 they reported the isolation of an active protein from an extract of giant ragweed pollen.[50] It contained 13.52 per cent nitrogen, 50.37 per cent carbon, and 6.40 per cent hydrogen and gave a negative Molisch test. They also isolated an active material of similar composition from an extract of short ragweed pollen.[12] In subsequent publications[51-53] Stull and associates reported the fractionation of extracts of ragweed

pollen by precipitation with ammonium sulfate. One of the active fractions contained 12 per cent nitrogen and appeared to be free from carbohydrate, while a second fraction contained only 2 per cent nitrogen and a considerable quantity of carbohydrate.[52] They also described the preparation of an active alkali-soluble protein fraction (glutelin) from ragweed pollen.[53] The active material in each of the fractions was found to be nondialyzable and, therefore, was considered to be a protein by these investigators.

Other evidence in favor of the protein nature of the allergens was presented by Cohen and Friedman.[55] They obtained active fractions from a pollen extract by fractional precipitation with ammonium sulfate. Freezing and thawing of these preparations resulted in separation of a pigment from unpigmented protein-like materials which were allergenically active.

Bukantz and his associates[56, 57] studied the dialyzable and nondialyzable components of ragweed pollen extract. The nondialyzable residue contained most of the allergenic activity of the original extract and displayed properties characteristic of proteins. After hydrolysis, the nondialyzable residue was found to contain fifteen amino acids as well as arabinose.

Recently Goldfarb and colleagues[58] isolated, by a series of alcohol precipitations and extractions with water, an allergenically active fraction (Trifidin A) from giant ragweed pollen. This appeared to be a single entity, chromatographically, electrophoretically, and immunologically as judged by agar gel diffusion techniques. Trifidin A was shown to be a chemically complex material; the hydrolysate gave a positive ninhydrin stain and contained arabinose and another unidentified reducing substance. The solution of Trifidin A in water, on standing in a refrigerator for about a month, was shown to be degraded into at least three components as revealed by the Kolin electrophoretic technique.[59]

EVIDENCE IN FAVOR OF THE NONPROTEIN NATURE OF THE ALLERGEN

Until the middle 1920's, it was widely accepted that the allergenically active substance in ragweed pollen was a protein. Then Grove and Coca[60, 61] announced that they had been able to eliminate all nitrogen from a pollen extract without causing a diminution in its allergenic activity. The extract was digested with trypsin and subjected to exhaustive dialysis. The dialyzed residue was found to be just as allergenically active as the original extract, although no nitrogen attributable to the pollen extract could be detected. Black[34] repeated the experiments of Grove and Coca and obtained identical results. Black and Moore[62] subsequently reported that a protein-free preparation (negative biuret and xanthoproteic tests, no precipitation with picric acid and sodium tungstate) gave good clinical results when it was used in the treatment of allergic patients.

Long and Teller[63] obtained an ultrafiltrate of a pollen extract which did not contain protein but which possessed all the allergens present in the original extract as it was capable of neutralizing sites to the whole extract.

Abramson and co-workers[64-69, 77] isolated an active fraction from an aqueous extract of ragweed pollen by free electrophoresis. It was colorless, it did not coagulate on boiling, it did not precipitate with trichloroacetic, nitric, and sulfosalicylic acids, and it gave a negative Molisch test. They claimed this material was a polypeptide of high molecular weight.

Hecht and associates[17] observed that an extract of ragweed pollen maintained at pH 4.0 and 60° C. for one hour resulted in the formation of a precipitate. Only the precipitate was capable of giving a positive precipitin test with rabbit antiragweed sera. However, the supernatant was found to be allergenically as active as the original whole extract. The authors claimed that the precipitate was composed of high molecular weight material and the supernatant of low molecular weight material although they did not present any evidence to support their claims. Mosko and colleagues[15] repeated the experiments of Hecht and associates and extended the study to include more immunologic experiments. The material which did not precipitate on heating to 60° C. at pH 4.0 contained essentially all the allergenic activity of the extract, but it was incapable of eliciting anaphylactic shock in guinea pigs sensitized to whole ragweed pollen extract and of precipitating with rabbit antiragweed serum. The material which precipitated at pH 4.0 and 60° C. possessed slight allergenic activity, induced anaphylactic shock in guinea pigs sensitized to the whole extract, and precipitated with rabbit antiragweed serum. Roth and Nelson[16] demonstrated that they could separate the allergenically active material from the constituents capable of reacting with the rabbit antiragweed serum. They extracted ragweed pollen for about twenty days, and the extracting solvent was examined each day for allergenic activity, nitrogen content, and ability to precipitate with rabbit antiserum. The eighth extract no longer possessed allergenic activity, whereas all the extracts up to the twentieth were able to precipitate with rabbit antiserum. These investigators concluded that the allergenic activity is a property of the low molecular weight material only.

Rockwell[70-72] reported the isolation of active material from a pollen extract by precipitation with hydrochloride. The precipitated material, which contained about 90 per cent of the allergenic activity of the whole extract, had a minimum molecular weight of 4,500, as determined by its sulfur content, and had the properties of a high molecular weight polypeptide. The precipitated material was not dialyzable, gave a positive biuret reaction, did not lose any activity on digestion with pepsin, and was not coagulated by heat. In addition to peptide material, the precipitate also contained arabinose and a pigment which Rockwell identified as isorhamnetin. From his data, Rockwell postulated that each allergen molecule consisted of one molecule of isorhamnetin, one molecule of arabinose, and two polypeptide chains containing a total of twenty-eight amino acids. The material precipitated with hydrochloride also gave good therapeutic results when used instead of whole extract in the treatment of allergic patients.[71-73] Subsequently, Rockwell reported that the allergenically active material precipitated with hydrochloride could be separated

into five active fractions by fractional precipitation of the whole extract with acid and salt solutions.[74] All the fractions were of different composition, and their minimal molecular weights varied from 650 to 4,500 as calculated from elemental analyses. All the fractions contained pigment, polypeptide, and carbohydrate components.

Brown and Benotti[75] treated ragweed pollen extract with decolorizing carbon (norite A) at pH 6.6 and found that the supernatant after centrifugation was very active by skin test although the charcoal had taken up over 60 per cent of the total nitrogen of the extract. The supernatant gave a negative xanthroproteic test but positive Molisch and ninhydrin tests and was found to contain free amino groups. Brown and Benotti[76] also precipitated the ragweed pollen extract with phosphotungstic acid and found that only 50 per cent of the nitrogen was precipitated. The supernatant did not contain detectable protein, and it produced large reactions when injected into the skin of allergic persons. This preparation also produced reactions, however, though of lesser intensity, in the skin of normal, nonallergic persons.

In 1948 Robbins and associates[31] described the isolation of a heat-stable, allergenically active substance from an aqueous extract of the pollen. The extract was boiled for one hour at pH 4.0, which resulted in precipitation of the protein. The active supernatant was absorbed on colloidal aluminum hydroxide from which the active material was eluted with M/15 phosphate buffer, pH 7.4. The active material was then precipitated with alcohol at 0° C. The isolated material contained a carbohydrate component (composed of hexose, pentose, and hexuronic acid), flavonol pigment, and a component referred to by Robbins and co-workers as a "protein component." This latter constituent, on hydrolysis with hydrochloride yielded fourteen amino acids (arginine, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophane, tyrosine, and valine). This isolated material was allergenically more active than the whole extract when compared on an equal nitrogen basis. Since the material was dialyzable and did not precipitate with either picric or trichloroacetic acid, it is possible that the "protein component" was a polypeptide.

More recently, we[117] isolated an allergenically active peptide from short ragweed pollen. The water-soluble pollen extract (WSR) was heated at 60° C. for fifteen to twenty minutes. This resulted in precipitation of all detectable protein. The deproteinated WSR was allergenically as active as the whole WSR. This would indicate, therefore, that the protein is, at most, only slightly allergenic. The deproteinated extract was then fractionated by paper electrophoresis. One of the fractions after dialysis, referred to as AA_1-D, was found to be 1,000 times as active as the whole extract on an equal weight basis and was found to be composed of pigment and peptide material. Thus, the injection of 10^{-7} mg. of this fraction into the skin of an allergic person elicited a reaction as large as 10^{-4} mg. of the whole extract of WSR. The allergenic activity appeared to be associated solely with the peptide component(s). Chromatographic analysis of this material after hydrolysis with hydrochloride

revealed the presence of eight amino acids: lysine, arginine, glycine, glutamic acid, hydroxyproline, alanine, valine, and norleucine. This fraction had a sedimentation coefficient of 1.2 S in saline at pH 7.0. As mentioned in the previous section, the fraction Trifidin A of Goldfarb and associates[58] was also shown to contain peptide component(s). It would be most interesting to establish the chemical composition of the latter peptide(s) and the allergenic similarity, if any, between AA_1-D and Trifidin A.

ALLERGENICITY OF THE CARBOHYDRATE CONSTITUENTS OF RAGWEED POLLEN EXTRACT

The finding of Grove and Coca[60, 61] that all the nitrogen of a pollen extract could be eliminated without any accompanying loss of cutaneous activity influenced many investigators to turn their attention to the possibility that the allergen(s) in ragweed pollen might be a carbohydrate. In 1931, Black[78] isolated an active substance of carbohydrate nature by precipitation of the pollen extract with alcohol at pH 4.4. The active material did not precipitate with picric, tannic, and phosphotungstic acids, and the biuret, Millon, and xanthoproteic tests were negative. The Molisch test was positive. The material, when made up to a volume equal to that of the original extract, was more active than the latter. Hydrolysis of this carbohydrate fraction yielded 55 per cent reducing sugar, and the nitrogen content was 6 per cent. Black concluded that the material was not a protein but a complex carbohydrate. He did not explain what the nitrogen content of the material might represent. Conceivably, it could have represented up to 37 per cent protein or peptide (assuming a conversion factor of 6.25).

Caulfeild and co-workers[79] also isolated an active material rich in carbohydrate. The method used for its isolation was very harsh, and it is most surprising that the activity was not destroyed in the isolation process. The protein in the extract was precipitated at pH 4.2, and the carbohydrate was then precipitated with lead acetate at alkaline pH. The precipitate was dissolved by grinding it in cold, dilute, sulfuric acid. The lead sulfate was removed by centrifugation, and the excess sulfuric acid was removed by the addition of barium hydroxide. The material which remained in solution was dialyzed, reprecipitated with alcohol, redissolved in trichloroacetic acid, and finally reprecipitated with alcohol and dried. The final product contained only 1.5 per cent nitrogen and, after hydrolysis, yielded 58 per cent reducing sugar. It was active by both direct skin test and passive transfer.[79, 80] The investigators felt that protein could not have been present as a contaminant since this carbohydrate fraction was unable to elicit anaphylactic shock in guinea pigs sensitized to the whole pollen extract. However, if protein had been present in their preparation, which was not antigenic in the guinea pig, it would not have been detected. These investigators did not make any suggestion concerning the composition of the nitrogen-containing material present in the active fraction.

There has also accumulated evidence which suggests that the carbohydrate constituents of the pollen extract are not allergenically active.

Baldwin and colleagues[19] isolated several fractions from an aqueous extract of the pollen by fractional precipitation with alcohol. One of the fractions contained 60 per cent carbohydrate and only 1.4 per cent nitrogen and gave a negative ninhydrin test. However, this fraction was allergenically not as active as the other preparations, nor was it very antigenic in rabbits and guinea pigs. These workers found that the allergenic activity of the fractions diminished as the nitrogen concentration decreased.

In 1931, Stull and co-workers[50] isolated two active fractions from the pollen extract. One of the fractions contained a large amount of carbohydrate as well as some nitrogen, while the other consisted of protein and appeared to be devoid of carbohydrate as it gave a negative Molisch test. Since both fractions were able to neutralize sensitized sites to each other, these workers concluded that the carbohydrate was not active but that it had been contaminated with the protein which was allergenically active. Johnson and Rappaport[82] reported the isolation of a fraction composed largely of carbohydrate which was very active by skin test. However, they found that the active material could be precipitated by saturation of the carbohydrate fraction with ammonium sulfate, leaving the relatively inert carbohydrate in solution. They concluded that the active principle was not a carbohydrate.

Unger and associates[49] isolated a carbohydrate and a protein fraction from ragweed pollen. The carbohydrate fraction gave a very strong Molisch test and negative Millon and biuret tests. The nitrogen content of this fraction was 5.28 per cent. Sites passively sensitized with allergic serum failed to react to the carbohydrate solution after being neutralized to the protein fraction. On the other hand, all sites neutralized to the carbohydrate fraction gave positive reactions when challenged with the protein fraction. Unger and associates concluded that the carbohydrate in the pollen extract was not allergenically active.

ALLERGENICITY OF THE PIGMENT CONSTITUENTS IN RAGWEED POLLEN

Abramson and his fellow workers[64-69, 77] separated pigmented material from other constituents of the pollen extract by free electrophoresis and noted that the most rapidly migrating pigment was almost as active as the main, colorless, slow-moving fraction.[67, 77] Cohen and Friedman[55] succeeded in isolating pigment fractions which possessed allergenic activity. These fractions contained nitrogen, however, which would suggest that the fractions may have been contaminated with a small quantity of protein or peptide material.

Robbins and co-workers[31] reported the pigment constituent(s) in the pollen extract to be inactive, and a similar observation was made by Perlman,[84] who noted that very little allergenic activity was associated with the pigments when they were separated from the other components of the extract by paper chromatography.

Stevens and colleagues[83] noted that a pigmented material precipitated from an aqueous extract of the pollen on standing for several days at 4° C. Purification of this precipitate resulted in the isolation of the pigment isoquercitin

which, on hydrolysis, yielded two compounds—glucose and the pigment quercitin (a pentahydroxy-flavone). This preparation was allergenically inactive and contained no nitrogen. Preparations of isoquercitin obtained by mild methods of fractionation of the precipitate were allergenically active, but they also contained nitrogen, which would suggest that the activity may have been due to nitrogen-containing contaminants.

Goldfarb and associates[85] removed most of the pigment from ragweed pollen by extracting the pollen with 90 per cent methyl alcohol prior to its extraction with water. This pigment-deficient aqueous extract was allergenically as active as the aqueous extract prepared from the untreated pollen. These investigators felt that the pigment possessed no allergenic properties.

ALLERGENIC ACTIVITY OF THE LIPID FRACTION OF RAGWEED POLLEN

Milford,[86] in 1930, demonstrated that the ether-soluble fraction of the pollen possessed allergenic activity. The ether was evaporated from the ether extract of the pollen, leaving a residue with a semisolid consistency which was dissolved in a nonpolar solvent to exclude water-soluble material and Seitz filtered to remove pollen granules which might have been present in the extract. Qualitative tests showed the absence of nitrogen. The ether-soluble material gave positive cutaneous reactions in over 60 per cent of allergic persons tested, all of whom reacted to an aqueous extract of the pollen.

Moore and associates[87] dialyzed an ether-soluble extract against ether, using a membrane made of thin rubber, and found that the allergenic activity was not dialyzable. They did not state the pore size or permeability properties of the membrane. The dialyzed residue contained nitrogen and carbohydrate and was allergenically active in 50 per cent of allergic persons tested.

The ether-soluble extract obtained by Johnson and Rappaport[82] contained 0.2 to 0.3 per cent nitrogen and elicited positive skin tests in allergic patients.

DIALYZABILITY OF THE ALLERGEN

Unger and associates[88] observed that allergenically active material could dialyze through three different membranes (collodion, cellophane, and parchment) and that dialysis was more rapid with solutions at pH 3.2 than at pH 8.6.

Johnson and Rappaport[82] reported that the dialysate (they did not state the type of dialyzing membrane used) was active by skin test in patients, and Long and Teller,[63] using ultrafiltrate through cellophane membranes, observed that the ultrafiltrate contained less activity than the original pollen extract.

Allergenically active dialysates of pollen extracts were also obtained by Stone and co-workers[90] and by Bukantz and his group,[56, 57] who used Visking tubing as the dialyzing membrane. Stone and associates[90] found that 50 per cent of the allergenic activity in the extract was dialyzable, whereas Bukantz and associates[56, 57] observed that the dialysate was allergenically not as active as the dialyzed residue.

There have also been reports to the effect that the allergenically active material is not dialyzable. Grove and Coca[60, 61] and Black[34] showed that active material was not dialyzable through cellophane tubing, either before or after the whole extract had been thoroughly digested with trypsin. Spain and Newell[48] demonstrated that the degrees of diffusibility of allergenically active material through cellophane membranes depended upon the type of membrane used. A "thin" cellophane membrane (presumably a membrane of large pore size) allowed a large quantity of active material to diffuse through, whereas a very "dense" cellophane membrane (presumably a membrane of small pore size) allowed all the nonprotein nitrogen but not the allergenically active material to diffuse through. Stull and Sherman,[51] using Visking tubing, could find no activity in their dialysates of the pollen extract. Unfortunately, they tested their dialysates with the serum of only one allergic person.

More recently, Rockwell[70] reported that the active material obtained by precipitation of the pollen extract was nondialyzable.

The discrepancy in the observations pertaining to the dialyzability of the allergen(s) may be due to the fact that membranes of different composition and probably of different pore size were used by various investigators. Furthermore, the reports do not state whether treated* or untreated allergic persons were used in testing the dialysate for its allergenic activity. This is a very important factor to consider in view of the recent results obtained in our laboratory,[81] which demonstrated that the dialyzable constituents of pollen extract (using Visking membrane) were allergenic with respect to the skin and sera of treated allergic persons only. On the other hand, the nondialyzable fraction of the water-soluble pollen extract, which constitutes less than 5 per cent of the weight of the whole pollen extract, was allergenically active with respect to the skin and sera of both treated and untreated allergic persons. This finding suggests that the "desensitization" treatment with the pollen extract induces formation of new reagin(s) not produced spontaneously by allergic persons who have never been treated.

EVIDENCE FOR THE MULTIPLICITY OF ALLERGENS IN RAGWEED POLLEN EXTRACT

From the findings of these many investigations, one could arrive at the logical conclusion that there are several allergens of different composition in ragweed pollen. Much evidence has accumulated in support of such a view.

Milford[86] observed that reactions could still be elicited by injecting an aqueous extract of the pollen into passively sensitized sites previously neutralized to the ether-soluble fraction of the pollen. Moore and colleagues[49, 87] obtained similar results and claimed that there exist at least three distinct allergens in ragweed pollen.

Caulfeild and co-workers[79] obtained a number of active fractions from an extract of the pollen, and each was made up to the same volume as the original

*We refer to ragweed-sensitive patients who received at least one series of injections of ragweed pollen extract as treated or desensitized persons. Sera obtained from such persons are referred to as treated sera as compared to untreated sera obtained from untreated allergic persons.

extract. From results of cross-neutralization tests with the fractions on an equal volume basis, these workers concluded that there were several allergens in ragweed pollen. They also found that a fraction which was very active in the skin of one allergic person would sometimes be completely inactive in the skin of another.

Stull and associates[52] observed that two fractions obtained from an aqueous extract of ragweed pollen by precipitation with ammonium sulfate would not neutralize to each other. Cohen and Friedman[55] performed cross-neutralization tests with three active fractions isolated from a pollen extract by precipitation with ammonium sulfate at pH 4.0 and found that each fraction failed to neutralize to the other two. The results of their experiments suggested that ragweed pollen contained at least three different allergens.

Loveless and her fellow workers[91] separated a pollen extract by dialysis and then fractionated the dialysate by free electrophoresis. Results of cross-neutralization experiments with the fractions thus obtained suggested that ragweed pollen contained at least three allergens—two in the dialysate and a third in the nondialyzable portion of the extract. In a subsequent study, Loveless and Timasheff[92] fractionated a pollen extract by electrophoresis convection and were able to demonstrate the presence of at least two allergens by cross-neutralization tests.

In most of these studies, the allergenic fractions were tested on an equal nitrogen basis. It has, however, been shown[34, 48, 95, 115, 116] that there exists no correlation between the nitrogen content of a fraction and its allergenic activity. We and our colleagues[89] have shown in an extensive study that there is no rationale for comparing allergenic fractions on the basis of their nitrogen content and that such a comparison leads to fallacious conclusions. A variation of the cross-neutralization techniques was introduced by our group[89] and was shown to be more reliable. It was found that a sensitized site could be neutralized with solutions of the allergen(s) of varying concentration by injecting the site at daily intervals with the allergen. However, such a site was still capable of giving a reaction when subsequently challenged with the allergen solution in a much higher concentration. When the allergen solution was used above a certain threshold concentration, all the reagin in the sensitized site could be neutralized by a single injection with the allergen(s) and the site would no longer respond when challenged with the allergen, regardless of the concentration used. Thus, in the latter case the site had been desensitized and not just neutralized by the initial injection of the allergen. The term "desensitizing concentration" was defined as the concentration of an allergen or allergenic fraction (used in a Prausnitz-Küstner test) which will neutralize in one single injection a site of normal skin sensitized with an appropriate allergic serum. Using solutions of ragweed fractions isolated by paper electrophoresis in desensitizing concentrations, we showed that the water-soluble extract of short ragweed pollen contained a minimum of two allergens.[89]

EFFECTS OF HEAT AND HYDROGEN ION CONCENTRATION ON THE ALLERGENIC ACTIVITY

Gay,[93] in 1926, reported that heating an extract of ragweed pollen for as short a time as one minute at 100° C. produced a decrease in the allergenic activity. Although boiling the extract at 100° C. for one hour did not completely abolish the activity, autoclaving it for twenty minutes at a pressure of 15 pounds resulted in complete inactivation. Gay found that heat destroyed the activity of the pollen extract, regardless of whether the pH of the solution was 4.0, 7.4, or 8.4. Arbesman and Eagle[94] reported that 75 per cent of the allergenic activity of an aqueous extract of ragweed pollen was destroyed within fifteen minutes at 56° C., 90 per cent was destroyed within one hour, and more than 95 per cent was destroyed within four hours. Hampton and his fellow workers[96] also observed loss of allergenic activity in extracts heated at 56° C. for thirty minutes.

Stull and associates[95] reported that heating a neutral aqueous solution caused a diminution in activity, but they did not specify the length of time of heating or the temperature. They found that heating the whole pollen extract at 70° C. for one hour resulted in the destruction of most of the activity.[52]

However, evidence suggesting that heat does not destroy the activity of the pollen extract has been presented. Grove and Coca[61] and Black[34] showed that heating the extract for one hour at 56° C. or boiling it for ten minutes did not affect its allergenicity. Long and Teller[63] observed that heating an aqueous extract at 100° C. for one hour resulted in only a slight decrease in activity.

Rappaport[97] heated the whole pollen prior to its extraction at 140° C. for two hours. The allergenicity of the pollen extract, as determined by the size of the skin reactions which it induced in allergic persons and by its effectiveness in the treatment of hay fever, appeared to be unaffected by this treatment. Stull and colleagues[95] also claimed that heating the pollen in the dry state at 150° C. did not destroy its activity.

Stone and associates[90] heated the dialyzate of ragweed pollen extract for one hour at 100° C. and were unable to detect any loss of activity, and Robbins and co-workers[31] isolated a very active component from an extract of ragweed pollen after first boiling the extract for one hour at pH 4.0.

Loveless and associates[91] separated the aqueous extract of ragweed pollen into dialyzable and nondialyzable fractions. Heating the dialysate in a water bath at 100° C. for one hour had no observable effect on its allergenicity, whereas exposure of the dialyzed residue to 60° C. for one hour resulted in the loss of 50 per cent of its original activity.

Brown and co-workers,[98] in 1931, reported that the allergenic activity of a ragweed pollen extract was rapidly destroyed if kept in 0.1 N sodium hydroxide for a short period of time. Batchelder and Gonder[99] found that maintaining an aqueous extract of the pollen at 37° C. for four hours at any pH outside the range of 4.5 to 7.5 resulted in 75 per cent loss of activity and that the pH region for maximum stability of activity was approximately 5.5 to 6.5. It is interesting to note, however, that Rockwell[70-72] was able to isolate highly active material by precipitation with concentrated hydrochloride.

More recently, we[117] isolated from ragweed pollen an allergenically active peptide which lost all allergenicity after hydrolysis with hydrochloride. However, incubation of the material with hydrochloride at 25° C., a treatment which did not result in hydrolysis of the material, did not cause a diminution in allergenic activity. Similarly, there was no loss of activity when this fraction was heated at 100° C. at pH 7.0 for periods of as long as fourteen hours.

EFFECT OF ENZYME DIGESTION ON ALLERGENIC ACTIVITY

Enzymatic digestion of ragweed pollen extracts has been carried out on numerous occasions in attempts at defining the chemical nature of the pollen allergen(s). Although various enzymes, such as amylase, invertase, catalase, reductase, pectinase, phosphomonoesterase, adenosinetriphosphatase, and pyrophosphatase, have been shown to be present in ragweed pollen,[100, 101] their relationship to, or their effect on, the allergenic activity of the pollen extract has not been investigated.

Grove and Coca[60, 61] and Black[34] reported that digestion of either the whole pollen or the aqueous extract of the pollen with trypsin did not result in destruction of activity. On the other hand, Marsh and Huber[54] observed that digestion of ragweed pollen extract with pepsin, followed by prolonged dialysis, resulted in marked loss in allergenic activity which could not be accounted for by dialysis of the extract alone. They did find a small but constant amount of activity in the pollen extract unaffected by proteolytic digestion. Rockwell[103] suspended whole, defatted pollen in hydrochloric acid at pH 1 and digested the pollen with pepsin. The digestion of the pollen resulted in only a small loss of allergenic activity in the aqueous extract, which he claimed was due not to digestion of the active material but to a decrease in its solubility.

There have been a number of reports that the allergen can be absorbed through the gastrointestinal tract when the whole pollen is taken by mouth[104-106] and that it can be absorbed through the mucous membrane lining the nose and throat when the pollen is sprayed into the nostrils.[107, 108] These findings would suggest that the allergen is not destroyed by the enzymes present in the respiratory tract, stomach, and intestine. Hecht and associates[104] demonstrated that the absorption of the allergen through the gastrointestinal tract was dependent upon the acidity of the fluids in the stomach. They showed that a decrease in gastric acidity tended to facilitate absorption of the allergen from orally administered pollen extract, while an increase in gastric acidity prevented its absorption. They did not determine whether the latter effect was due to enzymatic digestion of the allergen or to alteration in the permeability of the membranes lining the digestive tract.

PHYSICOCHEMICAL STUDIES ON RAGWEED POLLEN EXTRACT

Chromatographic Studies on Ragweed Pollen Extracts.—Bukantz and associates[56, 57] fractionated the dialysate obtained from an aqueous extract of ragweed pollen by descending paper chromatography. In their initial study[56] they used lutidine as the solvent, which they later discovered was capable of

eliciting positive skin reactions in allergic persons although not in normal persons.* In a subsequent study, Bukantz and his group[57] used methyl cellosolve as the solvent and were able to separate the dialysate of the pollen extract into one carbohydrate and eight peptide fractions, all of which possessed allergenic activity. The free carbohydrates present in the dialysate were shown to be glucose and fructose. The dialyzed residue, after hydrolysis followed by chromatography, was found to consist of fifteen amino acids and arabinose. Most of the allergenic activity of the whole extract resided in the dialyzed residue. They were, however, unable to fractionate this material.

Perlman[84] separated the whole water-soluble extract of ragweed pollen by ascending paper chromatography and detected seven peptide, three carbohydrate, and two pigment bands. The chromatograms were not stained for protein. Most of the fractions eluted from the paper were found to be allergenically active. It is interesting to note that activity was also detected in segments of the paper devoid of pigment or stainable material.

Bernstein and associates[115] chromatographed a water-soluble extract of the pollen through a column of alumina. All the pigmented material was adsorbed at the top of the column and only colorless material, amounting to 60 per cent of the weight of the solids in the extract, passed through. Distilled water was used as the eluting agent, and the eluates were collected in 15 ml. quantities. Eleven such aliquots were collected, and most were found to be allergenically active by skin test.

Bookman and Wax[116] used ion exchange resins in an attempt to isolate the allergen from the pollen extract. They passed extracts of the pollen through columns of various resins (Dowex-2, Dowex-50, and Amberlite IRC-50) and eluted them with buffers of different pH. The majority of the eluates were allergenically active, but the investigators did not determine the composition of any of these fractions.

Electrophoretic Studies.—In a series of papers, Abramson and colleagues[64-69, 77] described the separation of ragweed pollen extract by free electrophoresis into as many as six pigmented components and a colorless material which appeared to be present in greater concentration than any of the other components. They were able to test both the colorless material and the most rapidly migrating pigment for allergenic activity by withdrawing them from the Tiselius cell at the termination of the experiment. The active, colorless material which was referred to by these investigators as the "immobile fraction" migrated very slowly to the positive pole (in phosphate buffer, pH 7.4) and had an electrophoretic mobility of 0.5×10^{-5} cm.^{2}/volt/second.[68] The diffusion coefficient of this material was found to be $1.7 \pm 0.5 \times 10^{-6}$ cm.^{2}/second.[64] From the electrophoretic pattern of the whole extract, they calculated that the immobile component constituted as much as 75 per cent of the total material in the extract. However, they did not take into account the possible

*Winkenwerder and colleagues[113] and Sherman[114] noted that many allergic persons reacted by skin test to nucleic acids and to purified purine salts, such as adenine sulfate and guanine chloride, the molecular structures of which bear some resemblance to that of lutidine.

contribution of the salt boundary which normally forms at the interface between the buffer and the solution and which also manifests itself as a stationary or immobile peak in the electrophoretic pattern. The immobile material was found to be present in both dialyzed and undialyzed preparations of the pollen extract. This finding was later substantiated by Loveless and her coworkers,[91] who also showed that the dialysate of the pollen extract had a greater proportion of electrophoretically immobile material than the dialyzed residue.

In 1943 Newell[109] repeated much of the work of Abramson and associates and extended the study by performing electrophoretic analyses on fractions obtained by various procedures from ragweed pollen. He found that none of the fractions isolated previously by other workers using precipitation methods was electrophoretically homogeneous. All fractions were complex mixtures of materials, and a stationary peak having almost no net charge was found in every preparation between pH 4.0 and pH 7.0. Newell pointed out that the stationary peak could not be correlated with allergenic activity, since it was also present in inactive preparations. The stationary peak could have been due, at least in part, to a salt boundary, although Newell made no mention of this possibility.

Although Newell was unable to prepare a homogeneous fraction from the pollen, Loveless and Best[111] reported the isolation, by electrophoresis convection, of a colorless, immobile fraction which appeared to be homogeneous and which was allergenically active. However, Loveless and Timasheff[92] later reported that this essentially immobile material separated into two components when subjected to prolonged electrophoresis in phosphate buffer at pH 5.5. Although Loveless and Timasheff separated the pollen extract into a number of fractions by electrophoresis convection, their conclusions are subject to serious revision, since more than 60 per cent of their preparation was dialyzable and the method of electrophoresis convection can be applied only to nondialyzable materials.

Stevens and associates[83] identified one of the rapidly migrating pigments as isoquercitin by comparing the electrophoretic pattern of the whole extract with that of a purified preparation of isoquercitin. They also analyzed the dialyzed residue of the pollen extract and found that it displayed a single, slow-moving peak (electrophoretic mobility = 0.6×10^{-5} cm.^{2}/volt/second) in buffer at pH 8.4.[110] They did not state the composition of the buffer. The diffusion coefficient of this apparently homogeneous material was 7.1×10^{-7} cm.^{2}/second in M/15 phosphate buffer, pH 7.4, which is about one-half the value obtained by Abramson and co-workers.

In 1954, Goldfarb and colleagues[85] reported the preparation of an aqueous extract which was obtained by extracting the pollen with water after it had been extracted with 90 per cent methyl alcohol. This preparation appeared to be less intensely pigmented than the aqueous extract of the untreated pollen. These workers claimed that this material was purified extract as compared to

the whole water-soluble extract of the pollen. Electrophoretic examination of this preparation in phosphate buffer, pH 7.4, revealed the presence of one main, slow-moving peak and a second peak which was a new component not present in the aqueous extract of the untreated pollen. More recently Goldfarb and co-workers[58] reported that their fraction Trifidin A behaved electrophoretically as a single component, but that on standing at about +2° C. for a month it was degraded into three distinct components. As previously mentioned, we[117] showed that the water-soluble extract of ragweed pollen (WSR) could be separated by electrophoresis on paper into four major allergenically active fractions. Each fraction was found to contain at least two chemically distinct constituents (pigment, carbohydrate, peptide, or protein) as well as materials with different sedimentation coefficients. When the fractions were examined by paper electrophoresis in various buffers, it was found that allergenic activity could be isolated from areas of the electropherograms which contained too small an amount of material for staining with the standard reagents used for demonstration of peptides, carbohydrates, pigments, or proteins. This finding was taken as evidence that the allergenically active constituent(s) might be present in exceedingly low concentrations in all these fractions. More recently,[117] after removal of protein from WSR, a peptide fraction was isolated by electrophoresis at pH 8.6 which was shown to consist of eight amino acids. This fraction, AA_1-D, was also shown to possess all the allergens present in WSR. During these studies it was observed that when each of the four fractions originally isolated by paper electrophoresis of WSR in borate buffer at pH 8.6 was subjected again to electrophoresis in the same buffer, the allergenic activity was not limited to the area of the electropherogram from which the fraction had been eluted but was found to have spread over the same region of the electropherogram as when the whole WSR was fractionated. One possible explanation for this behavior of the allergen(s) is that the material is continuously breaking down during fractionation. This may involve gradual dissociation of complexes composed of allergenically active constituent(s) bound to different carriers.

Ultracentrifugal Studies and Molecular Weight Determinations.—Sanigar[112] was the first to analyze ragweed pollen extract by ultracentrifugation, and he obtained a value of 1.0×10^{-13} second^{-1} for the sedimentation coefficient of his active extract. He suggested that this value indicated a molecular weight of less than 17,000.

Abramson and associates[64, 69, 77] found that the electrophoretically immobile and colorless material isolated from the pollen extract had a sedimentation coefficient of $1.5 \pm 0.2 \times 10^{-13}$ second^{-1}, which was identical to that of the whole extract. From a knowledge of its sedimentation coefficient and diffusion coefficient ($1.7 \pm 0.5 \times 10^{-6}$ cm.^{2}/second), they calculated a molecular weight of about 5,000 for this allergenically active material.

Stevens and colleagues[110] found that the dialyzed residue of the pollen extract had a sedimentation coefficient of 1.3×10^{-13} second^{-1} in phosphate buffer, pH 7.4, and on the basis of this value and the diffusion coefficient (7.1×10^{-7} cm.^{2}/second), they calculated a molecular weight of 17,800. On the other

hand, Loveless and Timasheff[92] estimated the molecular weight of the dialyzed residue to be between 2,500 and 4,000 on the basis of the sedimentation coefficient of 0.5×10^{-13} second^{-1} calculated for this active material in phosphate buffer, pH 5.5.

In only two of the above investigations, however, was the diffusion coefficient determined, and in none was the partial specific volume estimated. A molecular weight calculated on the basis of the sedimentation coefficient and the assumed values for the diffusion coefficient and partial specific volume is, at the very best, only a rough approximation of the true molecular weight.

Rockwell[74] calculated minimum molecular weights of 650, 960, 1,117, 1,168, and 4,800 for his five active fractions on the basis of data obtained by a method independent of the physicochemical properties of the materials. He calculated the concentrations of all the elements in each preparation and divided all the values by that of the element present in the lowest concentration. He thereby found the minimum number of atoms of each element which must be present in a molecule of each fraction. However, Rockwell had to make the assumption that the fractions were homogeneous and he offered no evidence that they were.

Recently, our group[102] performed ultracentrifugation experiments* on fractions isolated by electrophoresis of the pollen extract on paper. The sedimentation coefficients for the dialyzed residues of the fractions were much higher than for the undialyzed fractions. The dialyzed residue of one of the fractions was resolved into two peaks in the ultracentrifuge with sedimentation coefficients of 1.81 and 4.51 S. These findings served to demonstrate that the fractions were heterogeneous with respect to molecular weight. The sedimentation coefficient of the active peptide isolated by us[117] was 1.20 S. No attempt was made to calculate the molecular weight, however, since the diffusion coefficient and partial specific volume were not determined.

SUMMARY

The results reported by numerous investigators who have attempted over the last forty years to isolate and establish the composition of the allergen(s) in the pollens of short and giant ragweed are conflicting. None of the investigations reviewed has provided a satisfactory proof that the allergen(s) have been separated in a pure form.

It would seem that the following two studies represent the most advanced stages in the characterization of allergens in ragweed pollens: The work of Goldfarb and associates[58] has led to the isolation from giant ragweed pollen, of a fraction (Trifidin A) which was shown to be chemically, electrophoretically, and immunologically a single entity. On standing in the cold in distilled water, however, it was degraded into at least three components: a ninhydrin-stainable material, arabinose, and another reducing substance. We[117] have recently isolated, by use of paper electrophoresis, a highly allergenically active fraction

*Performed in borate buffer, pH 8.6, ionic strength 0.1.

from short ragweed pollen (AA_1-D), which was shown to contain a pigment and peptide(s) composed of eight amino acids: arginine, lysine, glutamic acid, glycine, alanine, hydroxyproline, valine, and norleucine. The pigment could be removed from this fraction without loss of allergenic activity. On the other hand, hydrolysis of the peptide(s) resulted in a complete loss of allergenic activity; therefore, it was concluded that the peptide(s) appeared to be the allergen(s) in short ragweed pollen.

Furthermore, it was shown that 10^{-7} mg. of fraction AA_1-D was allergenically as potent as 10^{-4} mg. of the whole water-soluble extract of ragweed pollen. In view of the extremely high biologic potency of fraction AA_1-D, and in view of the fact that allergenically active fractions isolated in previous studies all contained some nitrogen material, it may be suggested that the allergenicity of ragweed pollen is due to peptide(s), which may be complexed with other carriers, such as proteins, carbohydrates, or pigments.

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Cutaneous and Systemic Reactions to Human Plasma

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A BLOOD transfusion is as much an organ transplant as is a skin graft, although it is not usually considered as such. It would be of great interest and considerable practical significance to know whether blood plasma is capable of provoking a homograft response when transplanted to a member of the same species. The purpose of this study was to determine whether or not human plasma proteins possess the same properties of immunologic individuality which characterize skin and other tissue proteins.

It is well known that the iso-antigens associated with erythrocytes are of significance in the causation of transfusion reactions, and recently it has been suggested that leukocyte antibodies may be responsible for some allergic reactions to transfused blood.[1] It is generally assumed, however, that human plasma is nonantigenic for human recipients, in spite of several sporadic published reports of allergic reactions to transfused plasma.[2-8] Such reactions have been attributed to either the passive transfer of antibodies or the inadvertent administration of proteins to which the patient was already sensitive. Dameshek and Neber[9] found evidence for a plasma factor in allergic reactions to whole blood occurring in patients with various hematologic disorders. They advised the use of washed erythrocytes as a means of preventing these reactions. Whealing in human skin as a result of intradermally injected human plasma was reported by Levine and State.[10] These workers believed that the whealing reaction was due to major blood group incompatibility, but they did suggest that plasma factors may account for some allergic transfusion reactions. Maunsell[6] observed that when pooled human serum, dried and reconstituted, was transfused into atopic persons, urticaria occurred in 80 per cent, whereas nonatopic recipients gave no reaction.

In previous publications, Bliss and his co-workers[11, 12] reported on plasma incompatibility in dogs. They skin-tested dogs with the dog's own plasma and with plasma from several other dogs. (In this article, plasma from the recipient itself will be called autologous plasma, and plasma from a different individual of the same species will be called nonautologous plasma.) At the same time as the animals were skin-tested with autologous and nonautologous plasma,

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they were given an intravenous injection of Evans blue dye. (The dye is bound to the plasma proteins, and the degree of extravasation of dye into the skin at the site of injection serves as an index of capillary permeability.) A total of 350 plasmas were tested in 180 dogs. In no case was there any reaction to autologous plasma, but when nonautologous plasma was used a blued area of increased capillary permeability always appeared at the site of injection of nonautologous plasma. The same distinction between autologous and nonautologous plasma was easily demonstrated among purebred and inbred dogs and was found to be unrelated to canine erythrocyte types.[13]

Bliss and associates also found that in dogs rapid transfusion of nonautologous plasma from a single donor nearly always resulted in the development of urticaria in the recipient animals. No reactions occurred in dogs receiving similar transfusions of autologous plasma. There was considerable evidence to show that in dogs both the cutaneous and the systemic reactions to nonautologous plasma were due to histamine release.

The present investigation was designed to explore the possibility that similar manifestations of plasma incompatibility may occur in human beings.

METHODS AND MATERIALS

Subjects.—The subjects were all healthy men between the ages of 20 and 35 years. Persons whose skin showed a positive intracutaneous reaction to saline, as well as those who showed no reaction to histamine, were rejected as subjects. The incidence of clinical allergy among the subjects was about 10 per cent, which is approximately that of the general population.

Skin Testing.—Fresh heparinized plasma (0.75 mg. heparin per 10 ml. of whole blood), 0.1 ml., was injected intradermally through a 26 gauge needle at each site in the recipient's back. At the time the sites were placed, 100 mg. of Coomassie blue dye (Ayerst, McKenna & Harrison, Montreal) was injected intravenously into the recipient. The skin tests were made in duplicate and were read in thirty minutes by two observers. An area of blueing, 2 to 3 mm. in diameter, appeared in each site at the point of needle puncture. Any larger area of blueing was considered to constitute a positive test, and the diameter was recorded in millimeters. The intensity of color and the presence or absence of flare formation were also noted.

Collection of Plasma.—Donors were bled into sterile siliconized vacuum bottles containing 0.55 gram citric acid and 1.54 grams trisodium citrate in 30 ml. of distilled water. Approximately 500 ml. of blood were collected from each donor. The blood was centrifuged at 10° C. at 1,700 r.p.m. and then the plasma was removed by suction and transferred to sterile bottles for storage. The plasma was stored at -20° C. for one week before it was used.

Transfusion of Plasma.—The plasma was infused through a No. 90 polyethylene catheter inserted in the antecubital vein; 500 ml. was given over a period of thirty minutes. A Sigmamotor pump was used to ensure a uniform rate of administration.

Determination of Blood Volume.—Radioactive iodinated serum albumin (RISA), 10 μc., was given intravenously for each blood volume determination. Plasma samples were taken at five-minute intervals for twenty minutes following the injection of RISA. The degree of radioactivity in each sample was determined in a well type of scintillation counter, and the blood volume was calculated from the values thus obtained.[14]

EXPERIMENTS AND RESULTS

Cutaneous Reactions.—The plasmas of forty-seven donors were tested in the skin of twenty-three recipients. Each recipient was skin-tested with autologous plasma and one or more nonautologous plasmas. In every case the donor and recipient were of the same ABO and Rh blood types. It was found that if no intravenous dye were given it was difficult to distinguish between the blebs produced by autologous and nonautologous plasma. A flare surrounded some of the nonautologous blebs, but this was not found to be a reliable means of separation. On the other hand, when dye was administered, a clear-cut differentiation was possible in almost every case. Although a bleb of almost identical size was produced at both the autologous and the nonautologous sites, there was marked blueing of the nonautologous blebs. There was no blueing at the sites of injection of autologous plasma.

TABLE I. COMPARATIVE DIAMETERS OF BLUED AREAS IN PAIRED SKIN TESTS WITH HUMAN PLASMA

	NO. OF CASES	PER CENT
Autologous greater than nonautologous	0	0
Autologous equal to nonautologous	9	19
Autologous less than nonautologous	38	81
	47	100

A difference of more than 4 mm. in diameter between the blued areas produced by autologous and nonautologous plasma in the same recipient was considered to be significant. Such a difference is easily distinguished on visual inspection without measurement.

TABLE II. THE REACTION OF HUMAN SKIN TO INTRADERMAL HUMAN PLASMA

PLASMA	NUMBER OF TESTS	AVERAGE DIAMETER OF BLUED AREA (MM.)	STANDARD DEVIATION (MM.)
Autologous	23	2.9	0.3
Nonautologous	47	9.1	3.5

The results of skin testing with autologous and nonautologous plasma are summarized in Tables I and II. In 81 per cent of the cases the skin reaction to nonautologous plasma was clearly greater than the reaction to autologous plasma, while in 19 per cent of cases the blued areas were of approximately the same size. In no case was autologous plasma more reactive than nonautologous plasma. In all the cases where there was not a clear distinction between autologous and nonautologous plasma, there was a reduced response to intradermal histamine, which meant that the recipients had poorly reactive skin.

When the average diameters of the blued areas were compared, it was found that the average area of increased capillary permeability produced by autologous plasma was 2.9 ± 0.3 mm., whereas that produced by nonautologous plasma was 9.1 ± 3.5 mm. (Table II).

Systemic Reactions.—In order to explore the possibility that this cutaneous sensitivity to nonautologous plasma might be of importance in the production of transfusion reactions, six healthy young men were given a transfusion of 500 ml. of their own plasma, which had been removed on a previous occasion. In no case did any untoward reaction to autologous plasma occur. The same subjects were then grouped into three pairs, and each subject was given 500 ml. of plasma taken from the other member of the pair. In three of these six nonautologous transfusions, there were severe reactions (Table III). It should be emphasized that 500 ml. of plasma was given over a period of thirty minutes. This represents a larger quantity of plasma given at a more rapid rate than is customary in normal hospital practice. The recipients were also given an intravenous injection of Coomassie blue dye to accentuate the skin reactions which were tested at the same time. Control studies showed that the transfusion reactions were not caused by the dye.

TABLE III. ALLERGIC REACTIONS TO TRANSFUSED PLASMA IN PAIRED HUMAN SUBJECTS

	PAIR I (A+)		PAIR II (O+)		PAIR III (O+)	
Plasma donor	1	2	3	4	5	6
Plasma recipient	2	1	4	3	6	5
Diameter of recipient's skin reaction to recipient's plasma	3 mm.	3 mm.	5 mm.	4 mm.	3 mm.	2 mm.
Diameter of recipient's skin reaction to donor's plasma	10 mm.	9 mm.	15 mm.	9 mm.	3 mm.	12 mm.
Allergic reaction	+++	-	++++	-	+++	-
History of allergy in recipient	Yes	No	Yes	Yes	No	No

One reaction to nonautologous plasma will be described in detail. The subject, a healthy 27-year-old resident physician with a history of seasonal rhinitis, began to complain of itching and swelling of the upper lip twenty minutes after the beginning of the infusion of nonautologous plasma. There was no visible change in the skin except for a faint erythema in the flexural creases of the neck and axillae. Multiple urticarial wheals then appeared over the entire body. The regions of urticaria were stained blue by the dye which had extravasated from the capillaries. Sixty minutes after the beginning of the transfusion, the wheals about the face had become confluent and appeared as diffuse blue areas which were slightly raised and surrounded by a patchy erythema. There was similar involvement of the anterior chest wall and back. The subject at this stage complained of intolerable itchiness, chills, nasal blockage, and a feeling of stiffness of the face. His symptoms disappeared gradually following the administration of 50 mg. of diphenhydramine intramuscularly and 0.5 ml. of epinephrine 1:1,000 subcutaneously. Ten hours later, the angioedema of the face recurred and was accompanied in this instance by pain in the knees and ankles. Diphenhydramine and epinephrine again produced a gradual remission of symptoms.

The transfusion reactions in the other subjects were essentially similar, except that joint pain occurred only in the subject already described. Six additional subjects were given rapid infusions of 500 ml. quantities of plasmas pooled from three donors. This, of course, is a much smaller pool than is usual for pooled plasma. Four of these six recipients had allergic transfusion reactions of varying degrees of severity.

The total experience with the transfusion of autologous and nonautologous plasma is summarized in Table IV. In six autologous transfusions given under the conditions already described, there were no adverse reactions, whereas there were seven transfusion reactions in twelve nonautologous transfusions.

TABLE IV. ALLERGIC REACTIONS TO TRANSFUSED PLASMA

PLASMA	NUMBER OF TRANSFUSIONS	NUMBER OF REACTIONS	REACTION RATE (PER CENT)
Autologous	6	0	0
Nonautologous	12	7	58

The skin tests proved to be of no value in predicting which subjects would have reactions and which would not. There was no correlation between the size of the skin reaction to nonautologous plasma and the severity of the systemic reaction to the infusion of that plasma.

Blood Volumes.—Blood volumes were determined in each subject before and after the transfusion of plasma. It was found that in those persons who had transfusion reactions, the expected increase in blood volume did not occur, presumably due to leakage of fluid from the capillaries. In some cases there was even a net decrease in blood volume. Detailed results of the blood volume studies will be reported at a later date.

DISCUSSION

On the basis of these experiments, it would appear that, in human beings, the normally responsive skin of every individual will react to the plasma of every other individual but not to his own. The plasma antigens are thus somewhat different from the naturally occurring cellular antigens of the blood, in that no equivalent to the blood types has yet been discovered. Each person appears to possess his own distinctive pattern of plasma antigenicity. In this respect, the plasma antigens most resemble the antigens found in tissue transplantation studies. However, they differ from tissue antigens in their capacity to elicit an immediate type of reaction in contrast to the delayed type of response provoked by homografts.

Plasma antigens may account for some of the reactions occurring in otherwise compatible blood transfusions. If this hypothesis is correct, allergic reactions should occasionally occur with plasma transfusions. In a series of 10,000 infusions of pooled plasma reported by Miller and Tisdall,[8] there were allergic reactions in 1.05 per cent. Weinstein[15] reported an allergic reaction rate of 1 per cent in 1,500 transfusions of unpooled plasma. In our series, the allergic reaction rate was approximately 50 per cent. The high incidence of

reactions in our study is probably accounted for by the fact that large quantities of plasma were given rapidly. Thus, any tendency for allergic reactions to occur was accentuated by the method of administration.

In the past, reactions to plasma have been assumed to be due to the passive transfer of skin-sensitizing antibodies, or to allergens to which the recipient was already sensitive. This explanation would not seem to be valid in our cases, since the incidence of systemic reactions approached 50 per cent. It is most improbable that 50 per cent of the population is sufficiently sensitized to food or other allergens for urticaria to be produced in such a manner. The incidence of systemic reactions to nonautologous plasma was not higher in allergic persons. In fact, in the majority of cases in which systemic reactions occurred, there was no history of allergy in either the donor or the recipient.

The urticaria and failure of plasma expansion associated with the administration of nonautologous plasma are probably best accounted for by increased capillary permeability similar to that demonstrated on skin testing. The infused fluid presumably leaks from the blood vessels into the skin and tissue spaces. Animal experiments strongly suggest that the increase in capillary permeability caused by nonautologous plasma is due to the release of free histamine.[12] It is probable that a similar mechanism exists in human beings, but this hypothesis has yet to be proved.

In the treatment of shock and severe hemorrhage, blood or blood plasma may have to be administered rapidly. This use of blood is especially important in times of national emergency. As a result of this study, it would seem reasonable to assume that patients who have allergic reactions to rapidly administered plasma derive little or no benefit from the transfusion. It is possible that under these circumstances a blood substitute, such as dextran, might be both more effective and easier to use. It would also appear that, contrary to current surgical teaching, there may be considerable advantage in slowing down the rate of transfusion in those patients who are to receive large quantities of whole blood or blood plasma.

SUMMARY

1. The normally responsive skin of every person will react to the intradermal injection of plasma from every other person with a localized area of increased capillary permeability. There is no reaction to the recipient's own plasma.

2. Allergic reactions occur in approximately 50 per cent of persons given rapid transfusions of unpooled plasma taken from other persons. No reactions occur when the recipient receives his own plasma.

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EVIDENCE OF ALLERGY IN PATIENTS WITH CYSTIC FIBROSIS OF THE PANCREAS

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THE frequent association of asthma and cystic fibrosis of the pancreas is well recognized. Table I summarizes data on this association obtained from three large published series of cases.[1-3]

This asthma has generally been assumed to be due to obstruction of the bronchi by the abnormal viscid secretion characteristic of this disease and by secondary infection and edema. Squamous metaplasia of tracheal and bronchial epithelium due to vitamin A deficiency, consequent to pancreatic steatorrhea, may have played a role in some cases. Pneumonia, atelectasia, emphysema, cyanosis, rhinitis, sinusitis, and clubbing of the fingers have been prominent associated findings. Progressive pulmonary infection and insufficiency have caused death in many cases. The importance of distinguishing asthma due to cystic fibrosis of the pancreas from allergic bronchial asthma is obvious and has been emphasized by many.[3-5]

TABLE I. PUBLISHED DATA ON THE ASSOCIATION OF ASTHMA WITH CYSTIC FIBROSIS OF THE PANCREAS

AUTHOR	TOTAL NUMBER OF PATIENTS WITH CYSTIC FIBROSIS OF THE PANCREAS	PATIENTS WITH ASTHMA ASSOCIATED WITH CYSTIC FIBROSIS OF THE PANCREAS	
		NUMBER	PER CENT
Bodian[1]	116	13	11
Abbott and associates[2]	102	10	10
Derbes and associates[3]	68	33	49

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At The John Hopkins Hospital during the past three years we have observed six asthmatic patients who have had evidence of both atopic allergy and cystic fibrosis of the pancreas. They have presented many problems in differential diagnosis, management, and interpretation of pathogenesis. In order to define these problems more clearly, the following study was undertaken.

MATERIALS AND METHODS

We reviewed the records of 135 patients with cystic fibrosis of the pancreas studied by the pediatric service of The Johns Hopkins Hospital since 1939. All had satisfactory clinical evidence of the disease, and the diagnosis was confirmed in 132 by demonstrating one or more of the following: deficiency of duodenal trypsin, sweat chloride concentration exceeding 60 mEq. per liter, or autopsy evidence of the disease. Each record was carefully searched for evidence indicating that the patient might have had atopic allergy in addition to cystic fibrosis. For the purposes of this study, one or more of the following was accepted as evidence of allergy: hay fever, nasal polyps, eczema, urticaria, and characteristic skin test reactions to pollens and other inhalant antigens. Asthma, chronic rhinitis, and family history of allergy were not accepted as evidence of atopic sensitivity per se.

This was a retrospective study. The records were well documented, and it is probable that most instances of hay fever, nasal polyps, eczema, and urticaria were reported. Only nineteen of the 135 patients had skin tests, however, and these tests revealed characteristic reactions to pollens and inhalants in thirteen. Had more of the 135 patients received skin tests, more cases of atopic allergy might have been detected. This study was restricted to patients referred to the pediatric service at The Johns Hopkins Hospital, and these referred patients could differ from the general population of cystic fibrosis patients with respect to incidence of allergy and age.

By the criteria just outlined, nineteen cystic fibrosis patients had evidence of atopic allergy. Data on these nineteen allergic cystic fibrosis patients were analyzed and compared with similar data on the total group of 135 cystic fibrosis patients.

The high incidence of allergy in patients with asthma and cystic fibrosis prompted a search for cystic fibrosis among patients with a diagnosis of atopic bronchial asthma in the pediatric allergy clinic of The Johns Hopkins Hospital. The demonstration of an excessive concentration of sodium and chloride in the sweat of almost all patients with cystic fibrosis of the pancreas has permitted a single test for detecting such cases.[6] The recent introduction by Gibson and Cooke[7] of local pilocarpine iontophoresis for induction of sweating has allowed the sweat test to be done safely, rapidly, and without discomfort. By this method, sweating is induced in a small area by local iontophoresis of pilocarpine. Thereafter, sweat is collected for thirty minutes on a previously weighed filter paper disk which is covered with a plastic sheet to prevent evaporation. Weight and chloride concentration of collected sweat can be determined easily.

Dr. Lewis Gibson measured the concentration of chloride in the sweat of forty-seven patients of the pediatric allergy clinic with a diagnosis of atopic bronchial asthma, using the iontophoresis method described above. The results were compared with similar measurements obtained from sixty-four control and twenty-one cystic fibrosis patients.

RESULTS

In Table II the evidence of cystic fibrosis in the nineteen patients with cystic fibrosis and allergy is compared with that in the total group of 135 patients. The evidence is similar in the two groups.

TABLE II. COMPARISON OF THE EVIDENCE OF CYSTIC FIBROSIS IN THE GROUP OF NINETEEN PATIENTS WITH CYSTIC FIBROSIS AND ALLERGY WITH THAT IN THE TOTAL GROUP OF 135 PATIENTS WITH CYSTIC FIBROSIS

	GROUP OF PATIENTS WITH CYSTIC FIBROSIS AND ALLERGY		TOTAL GROUP OF PATIENTS WITH CYSTIC FIBROSIS	
	NUMBER	PER CENT	NUMBER	PER CENT
Total number	19	100	135	100
Family history of cystic fibrosis	6	32	40	30
Abnormal stools	17	90	129	96
Pancreatic enzyme deficiency demonstrated	15	79	100	73
Rectal prolapse	2	11	14	10
Meconium ileus	2	11	10	7
Respiratory disease symptoms	19	100	130	96
Pulmonary infiltrate	19	100	123	91
Emphysema	15	79	82	61
Wheezing	12	63	51	38
Clubbing of fingers	15	79	61	45
Rhinitis	18	95	106	79
Sinus x-rays obtained	10	53	28	21
X-ray evidence of sinusitis	10	53	25	19
Failure to thrive	18	95	129	96
Sweat chloride				
Not measured	12	63	103	76
Normal	1	5	1	1
Elevated	6	32	31	23
Died of cystic fibrosis	6	32	56	41
Autopsy evidence of cystic fibrosis	3		37	
No autopsy	3		19	

In Table III the evidence of allergy in the nineteen patients with cystic fibrosis and allergy is compared with that in the total group of 135 patients. The method of selection required that the allergic group contain all patients with nasal polyps, hay fever, eczema, urticaria, or positive skin reactions to pollens and other inhalants. The criteria for selection of the allergic group did not require the inclusion of patients with asthma or familial allergy, but nevertheless the incidence of both was higher in the allergic group.

The nineteen patients with cystic fibrosis and allergy may be divided into two groups. The first group (Table IV) consists of eight patients with objective clinical evidence suggesting respiratory tract allergy. Seven had nasal polyps. These caused sufficient obstruction to necessitate surgery in four cases, and histologic study of the excised material revealed typical findings.

TABLE III. COMPARISON OF THE EVIDENCE OF ALLERGY IN THE GROUP OF NINETEEN PATIENTS WITH CYSTIC FIBROSIS AND ALLERGY WITH THAT IN THE TOTAL GROUP OF 135 WITH CYSTIC FIBROSIS

	GROUP OF PATIENTS WITH CYSTIC FIBROSIS AND ALLERGY		TOTAL GROUP OF PATIENTS WITH CYSTIC FIBROSIS	
	NUMBER	PER CENT	NUMBER	PER CENT
Total number	19	100	135	100
Family history of allergy				
Parents or siblings	5	26	20	14
Other than parents or siblings	8	42	14	10
Asthma	12	63	51	38
Rhinitis	18	95	106	79
Nasal polyps	7	37	7	5
Hay fever	4	21	4	3
Eczema	7	37	7	5
Urticaria	3	16	3	2
Positive intracutaneous tests				
Pollens	7	37	7	5
Other inhalants	13	68	13	10
Foods	5	26	5	4
Bacteria	6	32	7	5
Negative intracutaneous tests	0	0	5	4
Intracutaneous tests not done	6	32	116	86

TABLE IV. CLINICAL DATA ON EIGHT PATIENTS WITH CYSTIC FIBROSIS OF THE PANCREAS AND ALLERGY WHO HAD OBJECTIVE EVIDENCE OF RESPIRATORY TRACT ALLERGY

	D. H.	R. S.	M. R.	S. P.	K. C.	B. G.	C. N.	B. M.
Age in years								
Last observation	19	18	20	18	11	16	12	12
Death	Living	Living	Living	Living	11	Living	12	Unknown
Family history of allergy								
Parents or siblings	0	+	0	0	+	0	0	0
Other than parents or siblings	+	+	0	0	+	0	0	0
Age in years at onset of								
Asthma	16	15	12	1	2	16	-	1
Rhinitis	4	3	5	1	1	7	1	1
Nasal polyps	10	9	12	-	10	7	6	8
Hay fever	11	14	-	<10	-	7	-	-
Eczema	-	-	-	3/12	-	7	-	-
Urticaria	-	14	5	0	-	-	-	-
Respiratory symptoms near animals	<14	-	5	<10	-	7	-	-
Intracutaneous tests								
Pollens	+	+	Neg.	+	+	+	ND	ND
Other inhalants	+	+	+	+	+	+	ND	ND
Foods	ND	ND	Neg.	ND	ND	+	ND	ND
Bacteria	+	+	+	+	+	Neg.	ND	ND

Four patients had seasonal hay fever associated with characteristic positive intracutaneous reactions to pollens. Desensitization therapy was given to all four, with apparent benefit to three. Four patients noted lacrimation, sneezing, coughing, and/or wheezing when they were near various animals, associated with characteristic intracutaneous reactions to the appropriate animal dander. Corticosteroid therapy was used to advantage to control asthma in two patients. It is important to emphasize that these were relatively old

cystic fibrosis patients and that nasal polyps, hay fever, and respiratory symptoms near animals were noted no earlier than 5 years of age and usually at 10 years of age or later. Six of these patients had other manifestations of allergy (urticaria, eczema, and positive skin tests), and three had a family history of allergy.

The second group of patients with cystic fibrosis and allergy contains eleven who had no objective clinical evidence of respiratory tract allergy (Table V). This was a younger group of patients, most of whom had not attained the age at which nasal polyps and hay fever had appeared in the previous group.

TABLE V. CLINICAL DATA ON ELEVEN PATIENTS WITH CYSTIC FIBROSIS OF THE PANCREAS AND ALLERGY WHO HAD NO OBJECTIVE EVIDENCE OF RESPIRATORY TRACT ALLERGY

	M. D.	R. H.	D. D.	P. G.	R. M.	S. B.	T. A.	M. B.	R. N.	L. B.	S. C.
Age in years											
Last observation	2	8/12	4	8	2	2	6	3	1	1	1
Death	Living	8/12	Living	Unknown	3	Unknown	Living	4	Unknown	1	Unknown
Family history of allergy											
Parents or siblings	+	+	0	0	+	0	0	0	0	0	0
Other than parents or siblings	+	+	0	0	0	+	+	0	+	0	0
Age in years at onset of											
Asthma	1/12	3/12	2	7/12	–	–	9/12	–	–	–	–
Rhinitis	1/12	3/12	1/12	7/12	4/12	8/12	3/12	1/12	1/12	10 1/12	5/12
Nasal polyps	–	–	–	–	–	–	–	–	–	–	–
Hay fever	–	–	–	–	–	–	–	–	–	–	–
Eczema	–	–	<1	–	7/12	–	–	1/12	2/12	6/12	6/12
Urticaria	–	–	–	–	–	–	–	–	–	–	–
Intracutaneous tests											
Pollens	Neg.	ND	Neg.	+	Neg.	Neg.	Neg.	ND	ND	ND	ND
Other inhalants	+	+	+	+	+	+	+	ND	ND	ND	ND
Foods	ND	+	+	+	+	Neg.	Neg.	ND	ND	ND	ND
Bacteria	+	ND	Neg.	ND	Neg.	Neg.	Neg.	ND	ND	ND	ND

TABLE VI. COMPARISON OF GENERAL DATA ON THE GROUP OF NINETEEN PATIENTS WITH CYSTIC FIBROSIS AND ALLERGY WITH DATA ON THE TOTAL GROUP OF 135 PATIENTS WITH CYSTIC FIBROSIS

	GROUP OF PATIENTS WITH CYSTIC FIBROSIS AND ALLERGY		TOTAL GROUP OF PATIENTS WITH CYSTIC FIBROSIS	
	NUMBER	PER CENT	NUMBER	PER CENT
Total number	19	100	135	100
Male	9	47	78	58
Female	10	53	57	42
White	18	95	131	97
Negro	1	5	4	3
Age in years when last observed				
1 or less	4	21	53	39
2 to 5	5	26	50	37
6 to 10	2	11	18	13
11 to 15	3	16	8	6
16 to 20	5	26	6	4

In Table VI general data on the nineteen patients with cystic fibrosis and allergy are compared with data on the total group of 135 patients with cystic fibrosis. Sex and race distribution were similar in the two groups. The allergic patients tended to be older; 42 per cent of the allergic group and only 10 per cent of the total group were 11 years of age or older.

Table VII shows the percentage of cystic fibrosis patients in a given age group with evidence of allergy. The age reported is that which obtained at the time the patient was last seen. It is evident that with increase in age there was progressive increase in incidence of cases of allergy.

TABLE VII. NUMBER AND PER CENT OF CYSTIC FIBROSIS PATIENTS IN A GIVEN AGE GROUP WITH EVIDENCE OF ALLERGY

	TOTAL NUMBER OF PATIENTS	NUMBER OF PATIENTS WITH EVIDENCE OF ALLERGY	PER CENT OF TOTAL NUMBER OF PATIENTS WITH EVIDENCE OF ALLERGY
Total	135	19	14
Age in years when last observed			
1 or less	53	4	8
2 to 5	50	5	10
6 to 10	18	2	11
11 to 15	8	3	37
16 to 20	6	5	83

CASE REPORTS

CASE 1.—The patient was R. H., a baby boy.

Family History.—The mother and the maternal grandmother had hay fever, and a maternal uncle and the maternal grandfather had asthma. A brother died in 1941, at the age of 18 months, of pulmonary infection and asthma. Postmortem examination was reported to have revealed evidence of tuberculosis. It was impossible to determine subsequently whether or not this brother actually had cystic fibrosis of the pancreas.

Present Illness.—The patient had frequent pale, foul stools from the time of his birth in March, 1948. By the age of 3 months, he had "rasping" respirations. By the age of 5 months, he was having frequent respiratory infections associated with cough, wheezing, and nasal obstruction. Symptoms improved temporarily following the administration of penicillin.

At the age of 7 months, he was seen by a prominent allergist in another city, who noted that the nasal turbinates were pale and swollen. X-ray examination revealed clouding of both maxillary sinuses. Examination of the chest revealed typical wheezing. Skin tests revealed positive reactions to house dust, cat dander, orris root, pyrethrum, egg, beef, veal, apricot, peach, orange, coffee, chocolate, peas, beets, spinach, tomato, and cottonseed. Skin tests were negative to tree pollen, grass pollen, ragweed pollen, wheat, barley, corn, rice, chicken, lamb, plum, prune, grapefruit, tea, Coca-Cola, carrot, celery, Irish potato, and yeast. A diagnosis of atopic asthma and allergic rhinitis with superimposed infection was made. The diagnosis of cystic fibrosis was not considered.

At the age of 7 months the patient was admitted to The Johns Hopkins Hospital. Pale, swollen nasal turbinates and wheezing were still present. Chest x-ray studies revealed widespread linear infiltrations through both lung fields. Cultures of stools, nose, and throat revealed *Pseudomonas aeruginosa.* The patient had trouble expectorating thick tenacious sputum. Despite therapy with penicillin, streptomycin, sulfadiazine, digitalis, and

bronchodilators, there was increasing evidence of pulmonary insufficiency and fever, and the patient died at the age of 8 months.

Studies of the duodenal enzymes during the patient's Johns Hopkins hospitalization revealed an almost complete absence of trypsin activity.

Autopsy revealed evidence of cystic disease of the pancreas with obstruction of the pancreatic ducts by inspissated secretion and scarring and atrophy of the acinar tissue. There was evidence of acute purulent bronchitis and fresh and organizing lobular pneumonia. Cultures of the lung revealed *Pseudomonas aeruginosa, Staphylococcus aureus hemolyticus,* and *Aerobacter aerogenes.* There was atresia of the cystic duct, as well as a mucocele of the gall bladder. There was evidence of vitamin A deficiency consisting of keratinizing metaplasia of the tracheal and bronchial epithelium, keratinized cysts of the base of the tongue, and keratinizing Hassall's corpuscles in the thymus.

Comment.—This case clearly demonstrates how the diagnosis of cystic fibrosis of the pancreas may be overlooked in the presence of a strong family history of allergy, bronchial asthma, morphologic evidence suggesting allergic rhinitis and sinusitis, and positive skin tests. The presence of steatorrhea, pulmonary infiltrates, and respiratory symptoms should have suggested the diagnosis of cystic fibrosis.

CASE 2.—This patient was D. H., a white girl.

Family History.—A maternal uncle of the patient had urticaria following ingestion of aspirin, and a paternal cousin has asthma. There was no family history of cystic fibrosis of the pancreas.

Present Illness.—The patient had pneumonia at the age of 2 but was otherwise essentially well until 1942, when she was 4 years old. Then she began to have frequent, frothy, foul-smelling stools. Prolapse of the rectum occurred. A low-fat diet, vitamins, and pancreatin were given, and improvement in these intestinal manifestations ensued. Duodenal enzymes were not measured.

At the age of 4, the girl also began to have chronic cough and nasal discharge and frequent respiratory infections. The tonsils and adenoids were removed at the ages of 4 and 8, respectively. Windows were inserted in the maxillary antra when the patient was 6 years old. These operations did not relieve her symptoms.

At the age of 6 years she was examined at the University of Virginia Medical Center and was found to be allergic to various inhalant antigens and foods. She was desensitized for two years, and a diet was imposed. Apparently no improvement followed.

Nasal polyps were detected when she was 10, and these were removed at the ages of 10 and 12 years.

At the age of 14, she was seen for the first time at The Johns Hopkins Hospital. Examination revealed nasal polyps and chronic maxillary sinusitis. Bilateral radical antrum and nasal polypectomy were performed, and histologic studies of the polyps revealed typical findings. Chest x-ray studies revealed emphysema and infiltrations in both lungs. The patient complained of symptoms of hay fever of three years' duration in May, August, and September, and intracutaneous tests revealed reactions to tree, grass, and ragweed pollen extracts. Nasal infections and bronchitis were more frequent when she had hay fever. She noted sneezing, itching eyes, and cough when she was near dogs, cats, rabbits, and horses and after exposure to house dust. Intracutaneous tests revealed typical reactions to extracts of the offending danders, dust, feathers, kapok, orris root, wool, and molds. Desensitization was given to extracts of ragweed pollen, grass pollen, tree pollen, dust, orris root, feathers, kapok, wool, horse dander, dog dander, mixed bacterial vaccines, and staphylococcus toxin, and her symptoms of seasonal hay fever and asthma improved in the ensuing years.

At the age of 17, the patient had her first episode of asthma. This occurred for three days at the start of the ragweed season and in conjunction with symptoms of hay fever. Adrenalin relieved the paroxysms.

When she was 17 she was again hospitalized at The Johns Hopkins Hospital. Submucous resection, nasal polypectomy, ethmoidectomy, and antrotomies into the sphenoid sinus and both maxillary sinuses were performed.

When she was 19 three episodes of pneumonia occurred, the first beginning in the early part of the ragweed season. During the last of these episodes, she was in The Johns Hopkins Hospital. The chest x-ray is shown in Fig. 1 and presents a unique combination of infiltration, emphysema, and cyst formation. Sputum culture revealed *Pseudomonas aeruginosa*. Sweat chloride was elevated to 80 mEq. per liter. The fingers were noted to be clubbed. She recovered slowly on combined antibiotic-prednisone therapy. During the next year she was maintained on prednisone therapy and antibiotics as indicated, and she gained weight, noted fewer respiratory symptoms, and was able to enter college.

Comment.—This case demonstrates well the association of chronic infection and atopic allergy in cystic fibrosis. Specific desensitization and prednisone seemed to be important adjuncts to therapy.

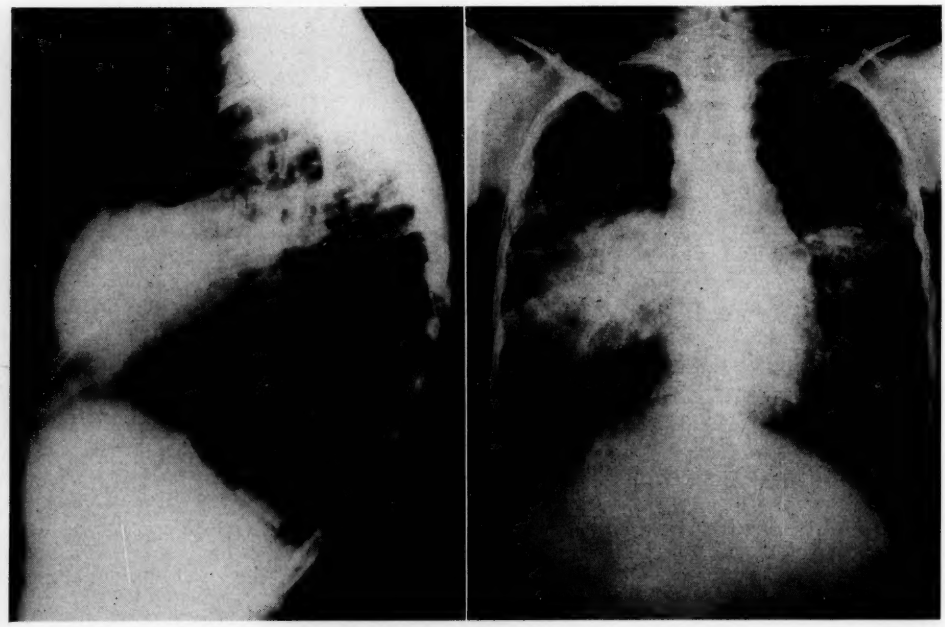

Fig. 1.—Case 2, Patient D. H. Roentgenograms of the chest, taken when the patient was 19 years of age, showing emphysema, infiltrations, and cysts.

Table VIII shows the concentration of chloride in the sweat of forty-seven patients with a diagnosis of atopic bronchial asthma. These results are compared with similar measurements obtained from control and cystic fibrosis patients. It is evident that the sweat chloride concentration of the asthmatic patients fell within the normal range; in no instance was it in the high range observed in patients with cystic fibrosis of the pancreas.

DISCUSSION

It is clearly apparent that patients with cystic fibrosis of the pancreas may have evidence of atopic allergy. The diagnosis of cystic fibrosis may be difficult in those with asthma, emphysema, chronic rhinitis, sinusitis, nasal

TABLE VIII. CONCENTRATION OF CHLORIDE IN THE SWEAT OF SIXTY-FOUR CONTROLS, FORTY-SEVEN PATIENTS WITH BRONCHIAL ASTHMA, AND TWENTY-ONE PATIENTS WITH CYSTIC FIBROSIS OF THE PANCREAS

	CONTROLS	ATOPIC BRONCHIAL ASTHMA	CYSTIC FIBROSIS OF THE PANCREAS
Total number of patients	64	47	21
Concentration of chloride in sweat (mEq. per liter)			
0-9	3	2	
10-19	31	30	
20-29	14	10	
30-39	8	2	
40-49	5	3	
50-59	1		
60-69	1		
70-79	1		1
80-89			5
90-99			3
100-109			6
110-119			3
120-129			2
130-139			1
Median	18.0 mEq./L.	14.0 mEq./L.	102 mEq./L.
Mean	23.0 mEq./L.	18.0 mEq./L.	101 mEq./L.

polyps, hay fever, urticaria, eczema, positive skin tests, and familial allergy. The aspects of the case suggesting allergy may cause one to overlook these signs indicating cystic fibrosis. However, such patients will usually have abnormal stools, pulmonary infiltrates, and clubbing of the fingers, and these supplementary clinical findings should lead one to suspect cystic fibrosis of the pancreas and to initiate confirmatory studies.

Management of these patients should include treatment of the allergic problem. Respiratory tract hypersensitivity to pollens, animal danders, and other inhalants may be treated through avoidance of causative antigens and specific desensitization with benefit to some patients. Corticosteroid therapy has been used effectively in some.

The absence of a sweat abnormality characteristic of cystic fibrosis in forty-seven patients with a diagnosis of atopic bronchial asthma indicates that cystic fibrosis patients with asthma had been previously excluded from the group by accurate differential diagnosis. This study suggests that "not many" patients with undiagnosed cystic fibrosis will be inadvertently included in the group of patients with atopic asthma treated in the average allergy clinic or practice. It is important to emphasize, however, that we have made such a mistake twice in the past three years.

The association of allergy and cystic fibrosis in the same patient may represent the chance coincidence of two unrelated disorders. This contention is supported by the observation that the incidence of familial allergy is higher in the allergic cystic fibrosis cases reported here than in total groups of cystic fibrosis cases reported here and elsewhere.[8]

An interrelationship between cystic fibrosis and allergy is suggested by the evidence indicating an increase in incidence of concomitant allergic disease with increase in age, and one may wonder whether there is something about cystic fibrosis that favors the development of allergy or something about allergy which favors survival in cystic fibrosis. The final proof that incidence of allergic disease increases with age in cystic fibrosis patients, however, demands methodical allergy studies on total cystic fibrosis populations and repeated studies on the same patient as he grows older. The data presented here, derived from a retrospective study of referred patients, must be regarded as suggestive only.

SUMMARY

Review of the records of 135 patients with cystic fibrosis of the pancreas revealed that nineteen had evidence of atopic allergy. The diagnosis of cystic fibrosis was easily overlooked in patients with classical manifestations of atopic allergy, such as asthma, nasal polyps, hay fever, urticaria, eczema, positive skin tests, and familial allergy. Optimal therapy for many of these patients included treatment of the allergic problem as well as the usual regimen for cystic fibrosis.

Forty-seven patients with atopic bronchial asthma had no evidence of the sweat chloride abnormality characteristic of cystic fibrosis of the pancreas.

The nature of the relationship between cystic fibrosis of the pancreas and atopic allergy is not clear.

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THE ROLE OF HISTAMINE AND SEROTONIN DURING ANAPHYLAXIS IN THE MOUSE

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SEROTONIN (5-hydroxytryptamine) and histamine are released into the blood plasma during anaphylaxis in the rabbit.[1, 2] Further studies have shown that serotonin is liberated mainly from blood platelets, while histamine appears to be released from both platelets and tissues.[3] During anaphylactic shock in the rabbit, platelets and leukocytes are trapped in the lungs and not in other tissues.[4] Consequently, the amount of serotonin and histamine in the lung increases suddenly after the intravenous injection of the antigen. These studies indicate that both amines participate in the toxicity noted during anaphylaxis in the rabbit.

The role of histamine during anaphylactic shock in the mouse has been considered to be of little importance. Several facts form the basis for this opinion. The mouse is extremely insensitive to the action of histamine[5-8] when compared to other animal species, particularly the guinea pig. The total histamine content of the normal mouse is much less than the amount required to elicit a toxic reaction in this animal.[5] Antihistaminic drugs which completely protect guinea pigs from the adverse effects of anaphylaxis have no protective action during the same reaction in mice.[9] On the contrary, Mayer and Brousseau[5] have shown that a significant increase in toxicity occurred when mice were treated with the combination of histamine and pyribenzamine or Benadryl as compared to treatment with histamine alone. Using the in vitro Schultz-Dale technique with the mouse uterus, Fink and Rothlauf[10] demonstrated that the anaphylactic reaction was not influenced by the presence of Benadryl. More recently, Fink[11] presented evidence to implicate serotonin as a possible factor causing contraction of the uterine muscle during antigen-antibody reaction and speculated that anaphylaxis in the mouse may be dependent on the release of serotonin.

Parventjev and Goodline[6] first showed that mice, after treatment with pertussis vaccine, became much more sensitive to histamine than normal control animals. Pittman[12] has also reported that increased sensitivity to histamine occurs in mice during respiratory infection with *Hemophilus pertussis.*

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Other investigators have found that treatment of a mouse with pertussis vaccine causes not only a marked increase in sensitivity to histamine[7, 13] but also a greater susceptibility to anaphylactic shock.[9, 14] However, in vitro studies with the isolated uterus from a mouse treated with this vaccine did not indicate any greater reaction to histamine or to antigen-antibody combination.[10] Subsequently, it has been reported that mice injected with pertussis vaccine also develop an increased, nonspecific sensitivity to a large number of agents.[15]

Recently, increased sensitivity to serotonin also has been found in mice treated with pertussis vaccine.[15-17] The reason for the increased sensitivity to histamine, serotonin, and other nonrelated agents after the administration of the vaccine is obscure.

The mechanisms which lead to the various manifestations of anaphylaxis in the different animal species are probably not the same. The principal shock organ during anaphylaxis is known in several of these species. However, the main site of reaction and of sensitivity in the mouse is unknown.

The present investigation was designed to study anaphylaxis in the mouse with particular emphasis on the possible relationship of histamine and serotonin to this phenomenon. The serotonin and histamine content in blood and tissues after injection of these amines and during anaphylaxis in mice treated with pertussis vaccine was also studied because of the probable relationship between the increased sensitivity to serotonin and histamine and to anaphylactic shock in these mice.

METHODS

A difference between various strains of mice in sensitivity both to anaphylaxis and to histamine has been shown.[18] For the present study, female white mice of a closely inbred strain were used exclusively. The females of this strain, as compared to the males, have a greater sensitivity to histamine,[7] and anaphylaxis is associated with a high per cent of deaths.

Mice of the same age, weighing 18 to 22 grams, were sensitized to horse serum by the method of Mayer and Brousseau.[5] Anaphylaxis was produced by the intravenous injection of 1 ml. of horse serum per 25 grams of mouse. Normal control mice showed no evidence of toxicity when this amount of horse serum was injected intravenously.

To study the increase in sensitivity to histamine and serotonin produced by pertussis vaccine, 0.15 ml. (9,000 million bacteria) of pertussis vaccine, phase 1, was injected intraperitoneally into each mouse. Normal control mice received 0.15 ml. of n-saline. Histamine and serotonin were injected intravenously five days later, at the time the experimental mice were considered most sensitive to these amines.[7, 8, 19] When anaphylaxis was to be produced, after treatment with pertussis vaccine, each mouse was injected with 0.85 ml. of horse serum and 0.15 ml. of pertussis vaccine. Control mice received the pertussis vaccine only. Fourteen days later, at the time the experimental

animals should be most susceptible to anaphylactic shock,[9] 0.5 ml. of horse serum per 25 grams of mouse was injected into each animal.

Immediately after respiration stopped, due to anaphylaxis or to injection of the amines, the chest of each mouse was opened. In every instance, the heart was found to be beating. Blood was obtained by direct aspiration from the heart. For each experimental animal, one control mouse was killed after the same interval of time following injection of the antigen, serotonin, or histamine. Control animals were anesthetized with ether to cause respiratory failure just prior to opening the chest to obtain blood. When ether was also used in the experimental animals, the analytical results for histamine and serotonin were similar to those obtained in experimental animals that were not exposed to ether. Siliconized glassware was used for drawing and handling the blood. The anticoagulant was heparin. Tissues were removed and pressed between porous paper to eliminate any excess blood. After careful weighing, the tissues were homogenized with 0.1 N hydrochloric acid. For measuring the histamine and serotonin changes, the same organs from six mice were pooled. These organs were then homogenized together. By this means, approximately 1 gram of lung tissue was obtained for each analysis. The first 10 cm. of the small intestine of the animals was used. Each whole mouse or mouse from which blood and lungs had been removed was homogenized with 0.1 N hydrochloric acid in a Waring Blendor and analyzed individually for histamine, serotonin, and also for 5-hydroxyindoleacetic acid (5-HIAA).

In order to study the possible in vitro release of serotonin, blood was obtained by cardiac puncture from mice sensitized to horse serum and incubated for thirty minutes at 37° C. with dilute horse serum. The procedure was the same as that used previously to show histamine and serotonin release from rabbit blood during in vitro antigen-antibody reaction.[1]

Serotonin and histamine were assayed by the method of Weissbach, Waalkes, and Udenfriend.[20] The 5-HIAA level was determined as follows by a modification of the urine method of Udenfriend and his co-workers.[21] Proteins were precipitated by the use of zinc sulfate and sodium hydroxide as described for the analysis of serotonin and histamine.[20] After centrifugation, to 5 ml. of the clear supernatant was added 1 ml. of 5 N hydrochloric acid in a glass-stoppered shaking tube. The clear solution was saturated with sodium chloride, 20 ml. of washed ether[21] was added, and the mixture was shaken for five minutes. After centrifugation, 18 ml. of the ether layer was removed, added to 1.2 ml. of 0.5 M phosphate buffer (pH 7) in a glass-stoppered tube, and shaken for three minutes. Following centrifugation, the ether was removed by suction. To 1 ml. of the aqueous layer in a small test tube, 0.3 ml. of concentrated hydrochloric acid was added. The fluorescence of the 5-HIAA in this solution was measured in an Aminco-Bowman spectrophotofluorometer at an activation wave length of 300 mm. and a fluorescence wave length of 540 mm. This method for measuring 5-HIAA in tissues is sensitive; its specificity has been shown previously.[22] Recovery of added 5-HIAA is 85 to 100 per cent.

RESULTS

The results in Table I show that during anaphylaxis in the mouse an increase in lung histamine occurs (lung A), with an associated increase in the amount of this amine in the blood. No distinct changes occurred in the histamine content of other organs. The amount of serotonin, on the other hand, did not appear to change significantly in the lung, blood, or other organs of the sensitized mice during anaphylaxis as compared to the control and normal

TABLE I. HISTAMINE AND SEROTONIN IN BLOOD AND TISSUES AFTER ANAPHYLAXIS*

TISSUE	NUMBER OF DETERMINATIONS	HISTAMINE (γ/GM. OR ML.) NORMAL AVERAGE (RANGE)	HISTAMINE (γ/GM. OR ML.) SENSITIVE AVERAGE (RANGE)	HISTAMINE (γ/GM. OR ML.) CONTROL AVERAGE (RANGE)	SEROTONIN (γ/GM. OR ML.) NORMAL AVERAGE (RANGE)	SEROTONIN (γ/GM. OR ML.) SENSITIVE AVERAGE (RANGE)	SEROTONIN (γ/GM. OR ML.) CONTROL AVERAGE (RANGE)
Lung A	8	0.8 (0.6-1.2)	4.2 (2.6-5.8)	1.0 (0.6-1.5)	2.8 (1.4-5.2)	3.4 (1.3-6.0)	3.2 (1.4-6.0)
Lung B†	2	— —	4.0 (3.9-4.2)	1.1 (0.8-1.4)	— —	4.0 (2.0-6.0)	3.7 (1.6-5.8)
Whole blood	6	<0.2‡ —	0.9 (0.2-2.0)	<0.3‡ —	3.2 (1.8-4.0)	2.8 (1.8-3.6)	3.4 (2.1-4.4)
Intestine	5	2.1 (1.6-2.3)	2.2 (1.9-2.6)	2.0 (1.5-2.2)	4.2 (3.5-4.5)	4.1 (3.2-4.3)	4.4 (3.6-4.8)
Brain	5	1.2 (0.9-1.4)	1.0 (0.6-1.4)	1.1 (0.9-1.1)	0.9 (0.6-1.1)	0.9 (0.6-1.2)	0.9 (0.7-1.1)
Liver	5	1.8 (1.1-2.3)	2.6 (1.8-2.8)	1.9 (1.2-2.3)	0.9 (0.6-1.1)	0.9 (0.5-1.2)	1.0 (0.5-1.4)

*Sensitized and control mice were injected intravenously with 1 ml. of horse serum per 25 grams of mouse. Histamine and serotonin were determined in blood and tissues. Each determination represents the value obtained by pooling the blood or tissue from six animals.

†Lung from mice which fifteen days previously had received pertussis vaccine and horse serum. Control animals received only pertussis vaccine. Anaphylaxis was produced by the intravenous injection of 0.5 ml. of horse serum per 25 grams of mouse.

‡The symbol < indicates that a reading was obtained on the Beckman spectrophotometer but was below the limits of its useful range.

levels for this amine. When horse serum was injected into the animals which had received pertussis vaccine plus horse serum fifteen days before, the same changes occurred as in those animals sensitized with horse serum alone. A rise in lung histamine was noted (lung B).

Table II gives the results of analyses for histamine, serotonin, and 5-HIAA in whole mice (after removal of the lungs and blood). No significant difference between the sensitized and control animals was found in the amount of

TABLE II. HISTAMINE, SEROTONIN, AND 5-HIAA CONTENT OF MICE AFTER ANAPHYLAXIS*

CONDITION	NUMBER OF DETERMINATIONS	γ PER GRAM HISTAMINE AVERAGE	HISTAMINE RANGE	SEROTONIN AVERAGE	SEROTONIN RANGE	5-HIAA
Sensitized to horse serum	10	6.7	(6.0-8.2)	1.3	(1.1-1.8)	<0.3†
Normal controls	10	6.2	(5.0-9.2)	1.4	(1.2-1.6)	<0.3†

*Anaphylaxis was produced by the intravenous injection of 1 ml. of horse serum per 25 grams of mouse. Normal controls were treated in the same manner. Each mouse, after removal of the lungs and approximately 1 ml. of blood, was homogenized in 0.1 N hydrochloric acid. The filtrate, after passage through gauze, was analyzed for histamine, serotonin, and 5-HIAA.

†The symbol < indicates that a reading was obtained on the spectrophotofluorometer, but that there was no peak in the fluorescence curve.

these substances remaining after the intravenous injection of the antigen. If histamine or serotonin is released during anaphylaxis, the amount liberated is either very small or is not metabolized quickly to other products. The latter would seem unlikely. Larger amounts of both histamine and serotonin than those found in the mouse have been injected intravenously and shown to be metabolized in the average time interval for fatal anaphylaxis to occur. Serotonin in the mouse is converted by monamine oxidase to 5-HIAA.[23] No significant amount of this acid was found, suggesting that little if any serotonin was liberated during anaphylactic shock.

The results in Table III show that serotonin is not released into the plasma when whole blood from sensitized mice is incubated with the antigen. Under the same conditions, however, when blood from sensitized rabbits is used, serotonin is readily liberated into the plasma as previously reported.[1]

TABLE III. PLASMA SEROTONIN*

ANIMAL	HORSE SERUM IN N-SALINE (PER CENT)	PLASMA SEROTONIN (γ PER MILLILITER)	
		SENSITIZED	NORMAL CONTROLS
Mouse	1	0.09	0.08
		0.08	0.08
		0.07	0.08
	10	0.08	0.09
		0.09	0.09
		0.09	0.09
		0.07	0.08
Rabbit	1	3.6 (2.5-3.8)†	0.5 (0.4-0.6)†

*Whole blood (2 ml.) from previous sensitized animals was incubated at 37° C. for thirty minutes with 50 per cent by volume of saline solution (1 or 10 per cent horse serum) of the antigen. After centrifugation, the diluted plasma was analyzed for serotonin and the values calculated on the basis of original plasma.

†The average value and the range for serotonin were determined after ten analyses. The rabbits were sensitized as indicated before.[1]

Tables IV and V show the results of the analyses for serotonin and histamine after the injection of these amines into mice which had been treated with pertussis vaccine. The amount of serotonin and histamine injected into the mice used for Table IV was less than the reported LD_{50} for normal mice; the amount of these amines used to obtain the data in Table V was equal to or greater than the LD_{50}. When the smaller amount of histamine was used, all of the experimental animals and none of the control mice died between nine and twenty-four minutes after injection. When the larger dosage of histamine was used all of the experimental animals died between ten and eighteen minutes after injection. A control mouse was killed at the exact time of death of each experimental mouse. The smaller amount of serotonin did not produce death, and both the experimental and control mice were killed after thirty minutes. The experimental mice showed distinct evidence of toxicity, while the control animals were unaffected. With the larger amount of serotonin, all the experimental mice died between twelve and seventeen minutes after injection, and also two of the control mice died between twelve and fifteen minutes after injection. The remainder of the control animals were killed at corresponding intervals in accordance with the time of death of the experimental mice.

TABLE IV. HISTAMINE, SEROTONIN, AND 5-HIAA AFTER PERTUSSIS VACCINE*

SPECIMEN	NUMBER ANIMALS	γ PER GRAM OR MILLILITER					
		HISTAMINE		SEROTONIN		5-HIAA	
		EXPERIMENTAL AVERAGE (RANGE)	CONTROL AVERAGE (RANGE)	EXPERIMENTAL AVERAGE (RANGE)	CONTROL AVERAGE (RANGE)	EXPERIMENTAL AVERAGE (RANGE)	CONTROL AVERAGE (RANGE)
Whole blood	18	2.6 (2.2-2.9)	1.1 (1.0-1.3)	5.3 (4.6-7.0)	5.8 (4.0-8.0)	—	—
Lung	18	7.5 (7.0-8.2)	4.7 (4.5-4.9)	11.2 (8.7-12.4)	7.8 (7.2-8.5)	—	—
Remainder of mouse	12	11.2 (10.8-11.3)	9.4 (8.4-11.0)	3.1 (3.0-3.4)	3.2 (2.9-3.4)	5.9 (5.0-6.5)	5.2 (4.5-6.1)

*Five days after the intraperitoneal injection of pertussis vaccine, the mice were given intravenously either histamine (11 mg. or 0.10 millimol per kilogram of base in the form of the dihydrochloride) or serotonin (26 mg. or 0.15 millimol per kilogram of base in the form of the creatinine sulfate). Control mice had received no pertussis vaccine.

TABLE V. HISTAMINE AND SEROTONIN AFTER PERTUSSIS VACCINE*

TISSUE	NUMBER ANIMALS	HISTAMINE (MG./ML. OR GM.)				SEROTONIN (MG./ML. OR GM.)			
		EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL	
		AVERAGE	(RANGE)	AVERAGE	(RANGE)	AVERAGE	(RANGE)	AVERAGE	(RANGE)
Whole blood	12	0.54	(0.52-0.56)	0.52	(0.48-0.58)	0.15	(0.13 -0.17)	0.13	(0.11 -0.15)
Lung	12	0.56	(0.52-0.60)	0.58	(0.56-0.60)	0.19	(0.18 -0.20)	0.17	(0.16 -0.17)
Brain	12	0.02	(0.01-0.02)	0.01	(0.01-0.02)	0.003	(0.003-0.004)	0.003	(0.002-0.004)
Liver	12	0.49	(0.44-0.54)	0.35	(0.34-0.36)	0.11	(0.10 -0.12)	0.11	(0.11 -0.12)

*Five days after the intraperitoneal injection of pertussis vaccine, the experimental mice were given intravenously either histamine (610 mg. or 5.5 millimols per kilogram of base in the form of the dihydrochloride) or serotonin (660 mg. or 3.7 millimols per kilogram of base in the form of the creatinine sulfate). Control mice which had not received pertussis vaccine were injected with histamine or serotonin.

The results in Table IV reveal that the histamine values were higher in the experimental than in the control tissues. The greatest difference was in the amount of histamine in the lung. The serotonin content of the experimental lung was also greater than the amount in the lung of the control animals. The amount of 5-HIAA in the whole mouse was essentially the same for the experimental and control mice. The data in Table V show little difference between the experimental and the control results except for the amount of histamine in the liver. The huge amounts of histamine and serotonin used probably obscure any small differences in tissue content of these amines as noted in Table IV. Of particular interest was the relatively low concentration of both histamine and serotonin in the brain of the experimental and control mice. This finding suggests a distinct blood-brain barrier for these amines in this animal species.

DISCUSSION

According to Mayer and Brousseau,[5] fifty-eight mice sensitized to horse serum were injected with 1 ml. of this antigen. All of the animals showed the characteristic picture of anaphylaxis, with an 89 per cent mortality. In

the present study of anaphylactic shock in the mouse, sixty sensitized mice were used. Each one showed distinct evidence of anaphylaxis. The mortality for the group was 80 per cent within ten to twenty-five minutes after the injection of horse serum. The total mortality probably would have been higher, but all animals that lived twenty-five minutes were killed at that time. The average time of anaphylactic death after the injection of antigen was eighteen minutes.

The picture of anaphylaxis in the mouse has been described by Mayer and Brousseau.[5] In the present work, the appearance of fatal anaphylaxis was typical and consistently the same. The principal effect noted was respiratory distress, generally developing five to ten minutes after intravenous injection of the antigen. Cyanosis was seen in those areas devoid of hair: paws, tail, ears, and the region about the nose and mouth. The respiratory difficulty became increasingly worse until breathing stopped. Frequently, there occurred convulsive movements which appeared to be associated with efforts to breathe rather than actual convulsions. When the thorax was opened immediately after complete respiratory failure, the heart of each mouse was found to be beating. The hearts from mice dying during anaphylaxis were contracted and small, in marked contrast to the hearts of the control animals. Much less blood could be obtained from the experimental mice compared to the amount readily obtained from the hearts of the nonsensitized control animals.

For the guinea pig, rabbit, and dog the principal shock organ during anaphylaxis is known. The lung in the guinea pig,[24, 25] the pulmonary vasculature and blood in the rabbit,[1, 24, 26] and the liver in the dog[24, 27] are the tissues which appear to be primarily involved during anaphylactic shock. For the mouse the end organ has not been known, and mice have been considered to be rather insensitive to anaphylaxis. However, depending on the strain and the method of sensitization, anaphylaxis in the mouse is associated with a high mortality. In this study, all the mice which died of anaphylaxis were in severe respiratory distress. During the fatal anaphylactic shock in the mouse, the lung appears to play a major role and is probably the principal shock organ involved for this animal species. The vascular system may be primarily affected.

As previously stated, histamine has been thought to be of little consequence during anaphylaxis in the mouse. Examination of the results found here shows that histamine rises in the lungs of mice dying as the result of severe anaphylactic shock. This finding suggests that histamine, or a histamine-like substance, is released from other tissues and bound in some manner in the lung. The amount of histamine released during anaphylaxis would be of little consequence to the lungs of a normal mouse. Antigen-antibody reaction, however, may convert the normally resistant lung into a tissue which is exquisitely sensitive to histamine. This could be accomplished directly or by

the binding of histamine in the lung or pulmonary vascular tree. The end result is reflected in the rise in the lung histamine concentration of the sensitized mice and the respiratory nature of their death. The fact that prior treatment with pertussis vaccine changes the mouse from an animal resistant to histamine and serotonin into one that is very sensitive to these amines indicates that distinct changes in sensitivity can occur in this species. In other animals, as the guinea pig and rabbit, pretreatment with pertussis vaccine has essentially no effect on sensitivity to histamine or serotonin. If anything, pertussis vaccine causes an increase in histamine tolerance in the guinea pig and rabbit.[28] In most animals, the action of histamine is completely blocked by antihistaminic agents. In the mouse, pyribenzamine and Benadryl actually increase sensitivity to histamine.[5] These findings demonstrate that the mouse normally reacts in a unique manner to specific physiologically active amines.

Serotonin has been implicated as the major toxic agent in mouse anaphylaxis on the basis of in vitro studies.[11] As with histamine, the total serotonin found in a single animal is considerably less than that amount which, if injected intravenously, would cause death. The data shown here do not reveal any significant differences between the amount of serotonin in the blood and tissues of the animals dying in anaphylactic shock and of the normal control mice. Incubation of blood from sensitized mice with the specific antigen did not produce a rise in the plasma concentration for serotonin in contrast to the effect obtained in rabbits.[1]

Preliminary in vitro studies with tissues from sensitized mice have also been made using a modification of the technique of Mongar and Schild.[29] Minced lung, brain, intestine, and skin have been suspended in Locke's solution and incubated with the antigen at 37° C. for thirty minutes. Analyses of the clear supernatant after centrifugation revealed a release of histamine from intestine and skin and of serotonin from intestine but no release of either amine from lung or brain. (The skin of mice has essentially no serotonin.) Analyses of the control samples, in which antigen was not used, show that the same amount of histamine and of serotonin, respectively, had been released from these tissues as from the experimental ones. Either the histamine in skin and intestine and the serotonin in intestine are chemically attached so that the bonds are more labile than those between these amines and lung and brain or the skin and intestine contain other substances capable of causing a release of histamine and serotonin under the circumstances of the experiment. Further studies are necessary to help clarify these problems.

The possibility has been suggested that those enzyme systems which destroy histamine and serotonin are inhibited by prior treatment of the mice with pertussis vaccine. This hardly seems correct in view of the marked variation in physiologic effect compared to the rather small difference between the pertussis-vaccine-treated mice and the control mice in the amount of these amines (Table IV). In addition, serotonin is converted to 5-HIAA by monamine oxidase.[23] No difference in the tissue content of 5-HIAA was found between the experimental and control animals, indicating equal enzyme activity.

It has been proposed that pertussis vaccine may alter the blood-brain barrier. When mice were injected with large amounts of histamine and serotonin, little difference between the pertussis-vaccine-treated mice and the normal control animals was found in the tissue content of these amines. The amount of histamine and serotonin was much lower in the brain as compared to other tissues and was essentially equal in the experimental and control mice. The data reveal that pertussis vaccine produces little if any change in the blood-brain barrier.

In view of the lack of any other concrete evidence to explain the action of pertussis vaccine, one might speculate that a protective substance is present in normal mice which blocks the effects of such compounds as histamine and serotonin. Because of the transient nature of the effect of pertussis vaccine, it may temporarily combine with or produce a change in the tissues to alter this protective substance. On the fifth day after injection of the vaccine, at the peak of sensitivity to histamine and serotonin, and end organs may not be protected from the effects of these amines. Perhaps pertussis vaccine acts by altering the permeability of specific tissues.

Malkiel and Hargis[9] have shown that mice injected simultaneously with pertussis vaccine and a small amount of horse serum (0.03 ml.) become maximally sensitive to this antigen fifteen days later, with 93 per cent deaths. Control animals, on the fifteenth day after being given only horse serum, had a mortality of only 4.5 per cent. However, if mice were given horse serum on the first day and pertussis vaccine on the eleventh day and challenged with 0.1 ml. horse serum on the fifteenth day, the mortality was only 11 per cent, despite the fact they were very sensitive to histamine. Two factors may be in operation in this situation: (1) the production of adequate amounts of antibody and (2) the increase in sensitivity. In view of these data, it would appear that pertussis vaccine may act as an adjuvant to induce an increase in antibody production in addition to its other properties. In the rat, however, Sanyal and West[30] found that pertussis vaccine did not produce an increase in precipitating antibody.

SUMMARY

Anaphylaxis was produced in female white mice and was associated with a high mortality. Death appeared to be due to respiratory failure. Histamine and serotonin analyses were done on tissues and blood after anaphylactic shock. No significant changes in serotonin content were found in in vitro or in vivo studies. Histamine, on the other hand, was found to rise in the lung of the sensitized animals during anaphylaxis. This finding suggests that histamine is released from some other site in the body and bound in the lung following antigen-antibody reaction. Thus, histamine appears to be of distinct importance in mouse anaphylaxis, while the role of serotonin is questionable. However, the entire phenomenon of anaphylaxis in the mouse is undoubtedly a very complex process, with many details still obscure.

Changes in tissue histamine and serotonin were also studied after injection of these amines into mice which had received pertussis vaccine. The possible mechanism by which pertussis vaccine alters the response to histamine and serotonin was discussed.

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QUANTITATIVE ANALYSIS OF ALLERGENS BY A PASSIVE TRANSFER METHOD AS DEMONSTRATED WITH FRACTIONS OF COTTONSEED ALLERGEN, CS-1A

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INTRODUCTION

THE isolation and properties of the principal allergen of cottonseed, CS-1A, have been described in a series of papers from this laboratory starting in 1939.[1] CS-1A was immunologically distinct from other allergens[2, 3] and antigens[4] in the cottonseed and was classified as a natural proteose.[4, 5] The protein nature of CS-1A was shown by chemical properties, isolations procedures, amino acid composition,[6] loss of activity on proteolysis,[7] and antigenicity.[8]

In a further study of CS-1A, dialysis and ion-exchange fractionation were used.[9] Eighteen subfractions were obtained from each of the dialysate (D series) and the endo (E series) fractions. Two fractions which represented maximum separation by these methods, (CS-13D)1A and (CS-13E)5F6, were completely free of each other as shown by an ion-exchange method of characterization and by a precipitin method.[9]

The purpose of this article is to evaluate the allergenic relationships of (CS-13D)1A, (CS-13E)5F6, and other important fractions from CS-1A and to describe a method for quantitatively comparing three properties of two allergenic fractions by the passive transfer technique. The properties are (1) the relative passive-transfer-inciting capacities, (2) the relative reagin-neutralizing capacities, and (3) cross-neutralization to determine whether or not the two fractions have the same specificity.

Walzer and Kramer,[10] in 1925, first adapted the passive transfer reaction of Prausnitz and Küstner[11] to diagnostic purposes. Walzer,[10, 12] Gay and Chant,[13] Sherman and Stull,[14] and other early investigators observed that allergen migrated from an injected sensitized site to uninjected sites if too many sites were injected or if challenging solutions which were too concentrated were used. More recently, Richter, Harter, Sehon, and Rose,[15] in studying the allergenic relationships of fractions isolated from ragweed pollen, encountered difficulty owing to migration of allergen in multiple-site testing.

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They also experienced difficulty in distinguishing nonspecific reactions when repeated, direct injections into sensitized sites were used to test for reagin neutralization. Attempts to eliminate or minimize the effect of migration of allergen have been made by reading reactions within five to twenty minutes after challenge and by using quantities of allergen close to the maximal combining capacity of reagins in the sites. Quantitative determination of reagin neutralization is affected more by migration than is the determination of reaction-inciting capacity because reagin neutralization can take place with a quantity of allergen too small to produce a visible reaction in an adjacent site. Control tests containing allergen also complicate the problem of migration in quantitative tests. The quantitative measurement of migration of intracutaneously injected CS-13 in passive transfer tests will be reported in another article.

The principal objections to comparison of allergenic fractions by multiple-site testing on a recipient and challenging by injection of allergen directly into the sensitized sites are as follows: (1) the recipient is caused considerable discomfort by the many sensitizations, challenges and control injections, (2) trauma of sensitized sites by direct injection, particularly where done repeatedly, makes difficult the distinction of specific from nonspecific reactions, and (3) interference caused by migration of allergen from sites injected with quantities of allergen in excess of that specifically bound by reagins. These objections are eliminated in the method described in this article.

The magnitude of the effect of migration on quantitative determinations of allergens may vary from negligible to significant with different systems studied and with different conditions of testing. In critical research studies, however, complete elimination of any possible influence of migration is essential. In the present method, the effect of migration was eliminated by using one test site on each recipient. The effect of trauma due to direct injection of sites and the need for control tests were eliminated by challenging sites by subcutaneous injection of allergen elsewhere in the body.[13, 14, 16-19] Feinberg and Bernstein[18] attempted to eliminate these objections by sensitizing with a series of twofold serial dilutions of the serum on a single recipient and challenging these sites by injection of allergen elsewhere in the body. Challenging of sites by subcutaneous administration of allergen required elimination of the excess allergen from the system before re-use of the recipient. The time interval that injected or injested allergen remains in the system has been determined by previous investigators. Levine and Coca[20] demonstrated timothy pollen allergen in the blood forty-eight hours after intravenous injection of a relatively large dose of timothy pollen extract; after seven days, detection was considered positive but questionable. Tuft[21] reported that traces of horse serum antigen were detectable in the blood seventeen days after intramuscular or intraperitoneal injection of 50 ml. of horse serum. Cohen, Ecker, and Rudolph[22] detected ragweed allergen in the blood twenty-four hours after blowing the pollen into the nose, but the

allergen was not detectable in eight of ten tests forty-eight and seventy-two hours later. Walzer and Walzer[23] observed that peanut allergen was eliminated from the blood forty-eight hours after ingestion of peanuts. It appears that time of retention of allergen in the body varies and depends on the amount given and the nature and probably the molecular size of the allergen. Ninety-six hours was allowed for the elimination of cottonseed allergen after subcutaneous injection.

ABBREVIATIONS USED

The symbols "γ," as used in the tables, and "mcg.," as used in the text, both refer to "microgram" (0.000,001 gram).

MATERIAL USED

CS-1A.—Cottonseed allergen, CS-1A, was isolated from depigmented, defatted cottonseed, as previously described.[5, 6]

CS-13.—Fraction CS-13 was prepared from CS-1A by precipitation with picric acid and recovery of active fraction by removal of the picric acid as described before.[7] Seventy-eight grams of CS-13, containing 17.0 per cent nitrogen (air-dried basis), was obtained from 235 grams of CS-1A.

Dialysis and Ion-Exchange Fractionation of CS-13.—The fractions obtained by ion-exchange fractionation of the dialysate (D series) and endo (E series) fractions of CS-13 have been described in detail.[9] Properties of fractions pertinent to this article are given in Table I. Demonstration by chemical and immunologic methods that (CS-13D)1A and (CS-13E)5F6 were free of each other has been described.[9]

TABLE I. COMPOSITION AND PROPERTIES OF PERTINENT COTTONSEED ALLERGENIC FRACTIONS

FRACTION	NITROGEN* (PER CENT)	CARBOHYDRATE* (PER CENT)	LOWEST CONCENTRATION OF FRACTION N GIVING PRECIPITIN REACTION† (γ/ML.)	MOLECULAR WEIGHT	REFERENCE
CS-1A	13.7‡	—			5-6
CS-13	17.0‡	—			7
(CS-13D)1A	17.5	3.1	None at 200	5 to 12 × 10^3	9
(CS-13E)1A	10.4	26.2	0.7	5 to 12 × 10^3	9
(CS-13E)5F6	20.1	0.45§	0.17	10 to 18 × 10^3	9
CS-13D	19.4	0.6	0.35		9
CS-13E	18.6	6.1	0.35		9

*Ash-water free basis except as noted.
†Cottonseed allergen rabbit antiserum was used.[9]
‡Air-dried basis.
§Estimated from nitrogen content.[26]

Reaginic Blood Serum.—A sufficient quantity of blood serum was collected from a cottonseed-sensitive subject (L.C.), so that all passive transfer tests were done with one lot. The serum was placed in sterile 15 ml. bottles, frozen, and

stored at –20° C. Serum was thawed and reserved at 5° C. at least one week before use. Once thawed, the serum was not refrozen since repeated freezing and thawing rapidly decreased its reagin content.

Test Subjects.—Recipients for passive transfer tests were volunteers at the District of Columbia Workhouse, Occoquan, Virginia,* who gave a negative skin test with CS-1A. Most of the recipients had a negative history of allergy. Cottonseed was eliminated from the diet, and recipients did not receive antihistaminic drugs during the tests.

Test Solutions.—Allergen fractions were dissolved in physiologic salt solution. Stock solutions were sterilized by heating at 100° C. for twenty minutes on three successive days. Dilutions of stock solutions were prepared aseptically with physiologic salt solution.

EXPERIMENTAL METHOD FOR COMPARING THE POTENCY AND CROSS-NEUTRALIZING CAPACITY OF TWO ALLERGENIC FRACTIONS BY PASSIVE TRANSFER

General Consideration.—A reaction which gave a discernible wheal in *exactly* sixty minutes was considered positive. The longest dimension of the wheal was measured and reactions were designated as follows: ±, questionable wheal; 1+, wheal up to 6 mm.; 2+, wheal from 7 to 12 mm.; 3+, wheal from 13 to 20 mm.; 4+, wheal over 20 mm.

The sensitivity of the skin varies significantly on different areas of the arms. Accordingly, sensitization was carried out in the same relative spot on opposite arms for the two phases of the test. Four pairs of sites were selected, two on the top of the biceps and two on the forearms. Designation and location of the sites were as follows: sites 1 and 2 were 87 and 37 mm., respectively, below the elbow bend on the anterior aspect of the forearm; sites 3 and 4 were 65 and 115 mm., respectively, above the elbow bend along the top of the biceps. Sites were located precisely with a celluloid T-square with 6 mm. holes appropriately spaced from the top of edge of the T-square which was laid on the outstretched arm with the elbow bend as reference point.

The interval required for elimination of CS-13 from the system after subcutaneous challenge was determined as follows: Eleven recipients were sensitized on the right arm, site 3, with 0.05 ml. of serum. Sites were challenged twenty-four hours later by subcutaneous injection of 32 mcg. of CS-13 nitrogen in the left arm. Twenty-four hours after this challenge, recipients were sensitized on the left arm, site 3, and challenged twenty-seven hours later by subcutaneous injection of 32 mcg. of CS-13 nitrogen in the right arm. Ninety-six hours after this challenge, recipients were again sensitized on the right arm, site 4, and challenged twenty-one hours later by subcutaneous injection of 32 mcg. of CS-13 nitrogen in the left arm. Readings were taken thirty and sixty minutes after each challenge. Results are shown in Table II. With the thirty-minute reading, the reactions were smaller in some

*The authors wish to express their appreciation to Donald Clemmer, Director of the Department of Corrections, and William F. Fleming, Superintendent, District of Columbia Workhouse, for their kind cooperation in permitting inmates to volunteer for these studies.

TABLE II. ELIMINATION OF CS-13 AFTER SUBCUTANEOUS INJECTION

RECIPIENT	TIME OF SENSITIZATION IN HOURS AFTER PRECEDING CHALLENGE*					
	30-MINUTE READING			60-MINUTE READING		
	ORIGINAL	24	96	ORIGINAL	24	96
B. C.	3+	3+	3+	3+	3+	4+
R. E.	2+	0	2+	2+	3+	3+
W. M.	3+	2+	3+	3+	3+	4+
M. P.	2+	0	2+	3+	3+	3+
W. B.	0	±	0	4+	3+	3+
W. W.	2+	3+	2+	3+	3+	4+
J. R.	4+	3+	3+	4+	4+	4+
J. L.	2+	±	2+	3+	2+	3+
R. C.	2+	0	3+	4+	4+	4+
F. C.	3+	0	3+	3+	3+	3+
C. T.	3+	3+	3+	3+	3+	3+

*Each challenge was made with 32 mcg. of CS-13 nitrogen administered subcutaneously.

cases with the twenty-four-hour interval after the first challenge with CS-13 than they were with the original test or with the ninety-six-hour interval test. With the sixty-minute reading, however, the reaction size was equal with the original and the twenty-four- and ninety-six-hour tests. These results showed that if allergen were retained for twenty-four hours after challenge, as indicated by the slightly lower thirty-minute readings, it was in extremely small amount.* To ensure that allergen was eliminated completely, a ninety-six-hour interval was allowed before continuing the second part of the experiment described below.

The method for comparing the passive-transfer-reaction-inciting, reagin-neutralization, and cross-neutralization capacities of two allergenic fractions is described with (CS-13D)1A and (CS-13E)5F6, as an example. Two groups (I and II) of eleven men each were used for the experiment which required eight meetings during eleven days. In order to eliminate, in so far as possible, the effects of individual variation in reactivity of recipients, both fractions were tested in equal quantities on each recipient in consecutive weeks. Data are shown in Table III, in which results of tests on both groups are shown in each column.

The method as described on a day-by-day basis follows:

First Day, Senitization.—Each recipient in Groups I and II was sensitized in site 3 on the right arm by intracutaneous injection of 0.05 ml. of L.C. serum.

Second Day (Twenty to Twenty-Four Hours After Sensitization) Reaction-Inciting Capacities.—

Group I: One milliliter of a solution containing one of a series of twofold serial dilutions of (CS-13D)1A—0.125 to 32 mcg. of (CS-13D)1A nitrogen per milliliter—was injected subcutaneously into the outer aspect of the upper left arm of each recipient, except for the control. Results are recorded in column *4*.

*Results of a similar unpublished experiment using 136 mcg. of nitrogen of the castor bean allergen, CB-1C, for challenging showed marked retention of allergen for twenty-four hours but complete elimination after ninety-six hours.

TABLE III. COMPARISON OF (CS-13D)1A AND (CS-13E)5F6 BY QUANTITATIVE PASSIVE TRANSFER ANALYSIS

RECIPIENT	GROUP NO.	NITROGEN* (γ/ML.)	(CS-13D)1A RESULT	(CS-13D)1A RESULT† 32 γN/ML.	(CS-13E)5F6 RESULT‡ 32 γN/ML.	(CS-13E)5F6 RESULT	(CS-13E)5F6 RESULT† 32 γN/ML.	(CS-13D)1A RESULT§ 32 γN/ML.
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
M. H.	I	0.125	0	3+	3+	0	4+	0
W. B.	II		0	3+	3+	0	3+	0
F. T.	I	0.25	0	3+	2+	0	3+	0
R. B.	II		0	0	2+	0	3+	0
P. C.	I	0.50	0	0	0	0	2+	0
G. H.	II		0	0	3+	0	3+	0
R. S.	I	1.0	0	0	3+	0	-‖	-‖
N. N.	II		0	0	3+	±	0	0
L. F.	I	2.0	0	0	3+	±	0	0
D. Q.	II		0	0	0	0	0	0
P. M.	I	4.0	0	0	2+	0	0	0
E. P.	II		0	0	+	3+	0	0
P. P.	I	8.0	±	0	3+	3+	0	0
B. W.	II		3+	0	3+	4+	0	0
C. S.	I	16	3+	0	2+	3+	0	0
J. B.	II		3+	0	3+	3+	0	0
H. R.	I	32	3+	0	3+	3+	0	0
J. M.	II		3+	0	3+	4+	0	0
A. C.	I	64	3+	0	3+	4+	0	0
L. H.	II		3+	0	0	4+	0	0

*(CS-13D)1A and (CS-13E)5F6 nitrogen used for determination of passive transfer inciting capacities as shown by results in columns *4* and *7*, respectively.

†Homologous neutralization by (CS-13D)1A and (CS-13E)5F6 nitrogen used in columns *4* and *7* to 32 mcg. of respective fraction. Results are shown in columns *5* and *8*, respectively.

‡Further reaction caused by 32 mcg. of (CS-13E)5F6 nitrogen in sites neutralized by (CS-13D)1A.

§No further reaction caused by 32 mcg. of (CS-13D)1A nitrogen in sites neutralized by (CS-13E)5F6 is shown in column *9*.

‖Not tested.

Group II: One milliliter of solution containing one of a series of twofold serial dilutions of (CS-13E)5F6—0.125 to 32 mcg. of (CS-13E)5F6 nitrogen per milliliter—was injected similarly into Group II. Results are recorded in column *7*.

Third Day, Neutralization.—

Group I: Each recipient was injected subcutaneously in the outer aspect of the upper left arm with 1 ml. of solution containing 32 mcg. of (CS-13D)1A nitrogen per milliliter. Results are recorded in column *5*.

Group II: Each recipient was similarly injected with 1 ml. of solution containing 32 mcg. of (CS-13E)5F6 nitrogen per milliliter. Results are recorded in column *8*.

Fourth Day, Cross-Neutralization.—

Group I: Each recipient was injected subcutaneously in the outer aspect of the upper left arm with 1 ml. of solution containing 32 mcg. of (CS-13E)5F6 nitrogen per milliliter. Results are recorded in column *6*.

Group II: Each recipient was injected similarly with 1 ml. of solution containing 32 mcg. of (CS-13D)1A nitrogen per milliliter. Results are recorded in column *9*.

Controls: The two recipients, who had not received any allergen since sensitization, were injected subcutaneously on the left arm, one with 1 ml. of (CS-13D)1A and one with 1 ml. of (CS-13E)5F6, containing 32 mcg. of fraction nitrogen, respectively. Control tests were always positive, which showed that the sites remained sensitive during the test interval.

Fifth, Sixth, and Seventh Days, Elimination of Allergen.—A four-day interval was allowed for elimination of allergen from the system before continuing the second part of the experiment.

Eighth Day, Sensitization.—All recipients in Groups I and II were sensitized with 0.05 ml. of L.C. serum in site 3 on the left arm (opposite arm from that used on the first day).

Ninth, Tenth, and Eleventh Days.—The procedure described for the second, third, and fourth days was repeated on the ninth, tenth, and eleventh days, respectively, except that the allergen fraction used on each group was reversed. For example, (CS-13E)5F6 was used on Group I and (CS-13D)1A was used on Group II on the ninth day and so on. Results are shown in appropriate columns of Table III.

RESULTS AND DISCUSSION

The reproducibility of the method of comparing passive-transfer-inciting and reagin-neutralizing capacities of allergens is shown in Table IV, where results of duplicate tests on four recipients at twofold concentration levels ranging from 0.125 to 32 mcg. of CS-13 nitrogen are given. The duplicate tests were made in consecutive weeks with a four-day interval between the end of one test and the start of the next. It is apparent in both incitation and neutralization that results of duplicate tests on the same subject and on different subjects with the same quantities of allergen are in excellent agreement. In incitation of reaction, ten of fifteen duplicates agreed perfectly, five varied by 1+ unit, and one varied by 2+ units. This latter test, V.A. at the 2 mcg. level, was the only one in which a positive reaction was obtained in one test and a negative reaction in a duplicate test.

No positive reaction was obtained with less than 2 mcg. of CS-13 nitrogen. One positive reaction was obtained with 2 mcg., and all reactons were positive with all levels from 4 to 32 mcg. of CS-13 nitrogen with all recipients, except for one ± reaction. In neutralization of reagins, nine of twelve duplicates agreed perfectly, two differed by 1+, and one differed by 2+ units. Agreement of results among the four individuals tested at each level was excellent. All sites were neutralized by 1 mcg. of CS-13 nitrogen, with three ± reactions at the 1 mcg. level. The reproducibility of the method in determination of both the passive-transfer-inciting and reagin-neutralizing capacities was

TABLE IV. PRECISION OF QUANTITATIVE METHOD FOR PASSIVE TRANSFER ANALYSIS WITH CS-13

RECIPIENT	INCITATION OF REACTION* CS-13 NITROGEN (γ/ML.)	INCITATION OF REACTION* RESULT I†	INCITATION OF REACTION* RESULT II‡	NEUTRALIZATION OF REAGINS§ CS-13 NITROGEN (32 γ/ML.) RESULT I	NEUTRALIZATION OF REAGINS§ CS-13 NITROGEN (32 γ/ML.) RESULT II
J. L.		0	0	4+	3+
P. M.	0.125	0	0	4+	4+
L. V.		0	0	4+	4+
R. P.		0	0	4+	2+
H. H.		0	0	3+	3+
W. S.	0.25	0	0	4+	4+
J. O.		0	0	4+	4+
J. B.		0	0	4+	4+
W. K.		0	0	3+	3+
J. A.	0.50	0	0	2+	2+
J. R.		0	0	4+	3+
O. G.		0	0	4+	4+
M. H.		0	0	0	±
A. B.	1.0	0	0	0	0
J. L.		0	0	±	0
P. M.		0	0	±	0
W. N.		0	0	0	0
V. A.	2.0	2+	0	0	0
L. F.		0	0	0	0
W. S.		0	0	0	0
C. H.		1+	±	0	0
W. W.	4.0	3+	4+	0	0
W. K.		3+	3+	0	-‖
J. A.		3+	3+	0	0
J. R.		3+	4+	0	0
O. G.	8.0	3+	4+	0	0
W. H.		0	-‖	0	-‖
V. A.		4+	4+	0	0
J. O.		4+	4+	0	0
J. B.	16	4+	4+	0	0
M. H.		3+	3+	0	0
A. B.		4+	4+	-‖	0
L. V.		3+	3+	0	0
R. P.	32	3+	4+	0	0
C. H.		4+	4+	0	0
W. W.		4+	4+	0	0

*Sites challenged by subcutaneous injection of 1 ml. of solution containing indicated quantity of CS-13 nitrogen per milliliter in outer aspect of upper arm opposite sensitized sites. Challenge was twenty-four hours after sensitization.

†Sensitization in site 3, right arm.

‡Sensitization in site 3, left arm, ninety-six hours after last challenge injection.

§Sites challenged by subcutaneous injection of 1 ml. of solution containing 32 mcg. CS-13 nitrogen per milliliter into outer aspect of upper arm opposite sensitized site. Injection was made twenty-four hours after the challenge test for determination of incitation of reaction.

‖Not tested.

judged to be such that serial dilutions, differing by only twofold, could be distinguished.

Comparisons of the passive-transfer-reaction-inciting, reagin-neutralizing, and cross-neutralizing capacities of (CS-13D)1A and (CS-13E)5F6 are shown in Table III. (CS-13E)5F6 was twice as potent as (CS-13D)1A in inciting reactions, because 8 and 16 mcg. of respective fraction nitrogen were required to

incite reaction with both recipients, with one recipient having reacted positively with 4 and 8 mcg. of nitrogen of the respective fractions. In contrast, (CS-13D)1A was twice as potent as (CS-13E)5F6 in homologous reagin neutralization because 0.5 and 1.0 mcg. of respective fraction nitrogen were required to prevent further reaction to 32 mcg. of homologous fraction nitrogen.

(CS-13D)1A did not neutralize sites to subsequent test with (CS-13E)5F6, but (CS-13E)5F6 neutralized to (CS-13D)1A, as shown in columns *6* and *9*, respectively, of Table III. If (CS-13E)5F6 had not neutralized sites to subsequent test with (CS-13D)1A, the evidence that their specificities were different would have been conclusive. However, Clarke,[24] Harkavy and Witebsky,[25] and Sherman and Stull[14] have reported that, with certain sera, unrelated allergens sometimes effected cross-neutralization of reagins. If nonspecific cross-neutralization has occurred in this case, the results would indicate that the specificities of (CS-13D)1A and (CS-13E)5F6 were different. For the present, an alternative interpretation that (CS-13D)1A possessed only part of the specificity determinant grouping found in (CS-13E)5F6 also must be regarded as possible.

Fraction (CS-13D)1A represented the maximum separation from (CS-13E)5F6 involving both dialysis and ion-exchange fractionation, whereas (CS-13E)1A represented maximum separation from (CS-13E)5F6 by ion-exchange fractionation alone.[9] Like (CS-13D)1A, (CS-13E)1A was completely separated from (CS-13E)5F6, as shown by an ion-exchange characterization curve like that in Fig. 4 of Ref. 9. Also, like (CS-13D)1A, (CS-13E)1A did not neutralize to (CS-13E)5F6, but (CS-13E)5F6 neutralized sites to subsequent test with (CS-13E)1A. These results are of interest because (CS-13D)1A gave no precipitin reaction with 200 mcg. per milliliter of (CS-13D)1A nitrogen, whereas (CS-13E)1A and (CS-13E)5F6 gave precipitin reactions with threshold concentrations of 0.7 and 0.17 mcg. of respective fraction nitrogen per milliliter with rabbit CS-13E antiserum.[9] Interpretation of these relationships is complicated because the carbohydrate contents of (CS-13D)1A, (CS-13E)1A, and (CS-13E)5F6 were 3.1, 26.2, and 0.45 per cent, respectively. Whether (CS-13E)1A gave a precipitin reaction because of its higher carbohydrate content than (CS-13D)1A or because the undialyzed (CS-13E)1A represented a larger protein molecule per se is not known. (CS-13E)1A was one-fourth as potent as (CS-13E)5F6 in reaction-inciting capacity and equally as potent as (CS-13E)5F6 in homologous reagin neutralization.

The potencies of (CS-13E)5F6, CS-13, and CS-1A were compared to ascertain whether the many steps involved in the preparation of CS-13 and (CS-13E)5F6 from CS-1A had significantly lessened the activity of these fractions. This was done by comparing both CS-1A and (CS-13E)5F6 to CS-13. CS-13 and CS-1A (Table V) were equally effective in inciting passive transfer reactions, as 4 mcg. of nitrogen of each fraction was required to cause reaction in both subjects (in both cases one positive reaction was produced by 2 mcg. of fraction nitrogen) as shown in columns *4* and *7*, respectively. In neutralization, however, CS-13 was four times more effective than CS-1A, because 0.5

TABLE V. COMPARISON OF CS-1A AND CS-13 BY QUANTITATIVE PASSIVE TRANSFER ANALYSIS

RECIPIENT	GROUP NO.	FRACTION NITROGEN (γ/ML.)*	CS-13		CS-1A	CS-1A		CS-13
			RESULT	RESULT† 32γN/ML.	32 γN/ML.‡	RESULT	RESULT† 32γN/ML.	32 γN/ML.‡
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
D. McN.	I	0.125	0	4+	0	0	4+	0
J. W.	II		0	4+	0	0	4+	0
A. E.	I	0.25	0	3+	0	0	4+	0
J. D.	II		0	3+	0	0	4+	0
D. McG.	I	0.50	0	0	0	0	0	0
H. D.	II		0	0	0	0	2+	0
S. P.	I	1.0	0	0	0	0	3+	0
E. A.	II		0	0	0	0	2+	0
D. A.	I	2.0	2+	0	0	0	0	0
F. S.	II		0	0	0	3+	0	0
A. D.	I	4.0	3+	0	0	3+	0	0
D. L.	II		3+	0	0	2+	0	0
A. C.	I	8.0	3+	0	0	4+	0	0
P. Y.	II		3+	0	0	3+	0	0
T. M.	I	16	4+	0	0	4+	0	0
L. F.	II		4+	0	0	3+	0	0
N. B.	I	32	3+	0	0	4+	0	0
G. R.	II		3+	0	0	3+	0	0

*Nitrogen of respective fractions used in columns 4 and 7 to determine passive-transfer-inciting capacities.

†Homologous neutralization with 32 mcg. of nitrogen of respective fractions.

‡Cross-neutralization.

and 2.0 mcg. of respective fraction nitrogen were required to neutralize the sites (columns *5* and *8*). CS-13 and (CS-13E)5F6 (Table VI) were essentially equal in inciting reactions, as CS-13 gave one positive with 1 and 2 mcg. of nitrogen, and (CS-13E)5F6 gave one positive at 1 mcg. and both positives with 2 mcg. as shown in columns *4* and *7*, respectively. Recipients H. D. and D. McG. were refractory. Both SC-13 and (CS-13E)5F6 were equally potent in reagin neutralization, as both required 1 mcg. of nitrogen to neutralize sites (columns *5* and *8*).

(CS-13E)5F6 represents 1.25 and 2.74 per cent of precursor fractions CS-1A and CS-13, respectively, on a nitrogen basis. Yet, the passive-transfer-inciting capacities of the three fractions were equal and the reagin-neutralizing capacities of (CS-13E)5F6 and of CS-13 were only four times as great as that of CS-1A. This observation substantiates our previous opinion that, essentially, CS-1A and CS-13 represent a mixture of closely related chemical components possessing a common allergenic specificity, because sites neutralized to highly purified (CS-13E)5F6 were also neutralized to CS-13 and CS-1A. Excluded from this consideration, however, are the possible different specificities of (CS-13D)1A and (CS-13E)1A which represent relatively minor fractions of CS-13. Immediate precursor fractions of (CS-13D)1A and (CS-13E)1A, (CS-13D)1 and (CS-13E)1, respectively, neutralized

TABLE VI. COMPARISON OF CS-13 AND (CS-13E)5F6 BY QUANTITATIVE PASSIVE TRANSFER ANALYSIS

RECIPIENT	GROUP NO.	FRACTION NITROGEN (γ/ML.)*	CS-13		(CS-13E)5F6	(CS-13E)5F6		CS-13
			RESULT	RESULT† 32γN/ML.	32 γN/ML.‡	RESULT	RESULT† 32γN/ML.	32 γN/ML.‡
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
D. McN.	I	0.125	0	4+	0	0	3+	0
J. W.	II		0	4+	0	0	4+	0
A. E.	I	0.25	0	3+	0	0	4+	0
L. F.	II		0	3+	0	0	3+	0
N. B.	I	0.50	0	3+	0	0	3+	0
G. R.	II		0	3+	0	0	3+	0
A. C.	I	1.0	1+	±	0	2+	0	0
D. L.	II		0	0	0	±	0	0
A. D.	I	2.0	2+	0	0	3+	0	0
J. D.	II		0	0	0	2+	0	0
T. M.	I	4.0	3+	0	0	3+	0	0
J. M.	II		4+	0	0	4+	0	0
L. B.	I	8.0	3+	0	0	3+	0	0
H. D.	II		0	0	0	0	0	0
D. A.	I	16	3+	0	0	3+	0	0
F. S.	II		3+	0	0	2+	0	0
D. McG.	I	32	0	0	0	0	0	0
D. R.	II		4+	0	0	4+	0	0

*Nitrogen of respective fractions used in columns 4 and 7 to determine passive-transfer-inciting capacities.

†Homologous neutralization with 32 mcg. of nitrogen of respective fractions.

‡Cross-neutralization.

sites to further test with (CS-13E)5F6 and (CS-13D)1 and (CS-13E)1 represented less than 10 per cent of CS-13. Fraction (CS-13E)5F6 appears to be approaching homogeniety because (1) it gave only one line of precipitate by the Ouchterlony method,[9] (2) it gave a precipitin reaction with the highest dilution, on a nitrogen basis, of any of the fractions,[9] (3) it is essentially carbohydrate-free as estimated from its nitrogen content, and (4) it sedimented uniformly in the ultracentrifuge.[9] Other criteria would be required for conclusive evidence of homogeniety.

SUMMARY

A quantitative method for comparing three properties of two allergenic fractions by the passive transfer technique is described. The properties are: (1) the passive-transfer-reaction-inciting capacities, (2) the reagin-neutralizing capacities, and (3) whether or not the specificities of the fractions are the same. The reproducibility of the method in the determination of both passive-transfer-inciting and reagin-neutralizing capacities was such that serial dilutions differing only twofold could be distinguished. The method eliminates the interfering effect of migration of allergen and the need for control tests. The method was demonstrated with subfractions, (CS-13D)1A

and (CS-13E)5F6, of the cottonseed allergen, CS-1A, which were completely free of each other. The significance of the observations made in evaluation of the separated allergenic components of CS-1A is discussed.

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QUANTITATIVE MEASUREMENT OF THE MIGRATION OF INTRACUTANEOUSLY INJECTED COTTONSEED ALLERGEN IN PASSIVE TRANSFER STUDIES

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INTRODUCTION

THE passive transfer technique is frequently used to evaluate allergenic properties of fractions isolated from natural sources or of modified preparations. Multiple sensitized site testing on the arms or back of a recipient usually has been used for these studies. Migration of allergen in excess of that combinable with reagins in the injected site and its possible invalidation of results has been recognized,[1] but quantitative measurements of migration with a well-defined allergen have not been described.

Need for a critical method of comparing the passive-transfer-inciting capacities, the reagin-neutralization capacities, and cross-neutralization relationships of isolated cottonseed allergenic fractions prompted development of a method which completely eliminated the problem of interference by migration. This method is described in our preceding article.[2] The literature on this subject was reviewed in that article and need not be repeated here.[2]

The object of this article is to describe the quantitative determination of migration of the cottonseed allergen, CS-13, in inciting passive transfer reactions and in neutralizing reagins in uninjected sensitized sites adjacent to challenged sites.

ABBREVIATIONS USED

The symbol "meg." refers to "microgram" (0.000,001 gram). The symbol "Mγ," used in the tables, refers to "millimicrogram" (0.000,000,001 gram).

MATERIAL USED

Fraction CS-13, test solutions, test subjects, and the LC cottonseed reaginic serum have been described.[2] The CN cottonseed reaginic serum was stored at 5° C.

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EXPERIMENTAL

Sensitization.—Each of twelve recipients was sensitized intracutaneously with 0.05 ml. of serum in two sites 50 mm. apart. Choice of site location is discussed in the preceding article.[2] For the LC serum test, the top of the left biceps was used; sites 3 and 4 were 65 and 115 mm. above the elbow bend, respectively. For the CN serum test, the anterior aspect of the right forearm was used; sites 1 and 2 were 87 and 37 mm. below the elbow bend, respectively.

TABLE I. INCITATION OF PASSIVE TRANSFER REACTION BY MIGRATION OF ALLERGEN

RECIPIENT	SITE*	CS-13 NITROGEN (Mγ)	LC SERUM†		CN SERUM‡	
			30 MIN.	60 MIN.	30 MIN.	60 MIN.
J. H.	I	1	2+	2+	2+	2+
	U	0	0	0	0	0
H. M.	I	2	2+	2+	2+	2+
	U	0	0	0	0	0
A. H.	I	4	3+	3+	2+	2+
	U	0	0	0	0	0
G. W.	I	8	2+	2+	3+	3+
	U	0	0	0	0	0
J. C.	I	16	3+	3+	3+	3+
	U	0	0	0	0	0
J. Ca.	I	32	2+	3+	2+	3+
	U	0	0	0	0	0
J. M.	I	64	2+	3+	3+	3+
	U	0	0	0	0	0
P. P.	I	128	3+	4+	4+	4+
	U	0	0	0	0	0
W. W.	I	256	3+	2+	3+	4+
	U	0	0	0	0	0
J. D.	I	512	4+	4+	3+	3+
	U	0	0	0	0	0
R. B.	I	1,024	3+	3+	4+	4+
	U	0	0	2+	0	0
H. T.	I	2,048	4+	4+	4+	4+
	U	0	±	4+	0	3+

*I = Injected site; U = uninjected site.
†Sites 3 and 4 were used, site 3 being the injected site.
‡Sites 1 and 2 were used, site 1 being the injected site.

Four days after completion of the test with the LC serum, the same recipients were sensitized for a similar test with CN serum. This interval between tests ensured elimination of excess allergen from the system from the previous test.[2]

Measurement of Reaction.—A reaction which gave a discernible wheal was considered positive. Reactions were measured, using the longest dimension of the wheal, and designated as follows: ±, questionable wheal; 1+, wheal up to 6 mm.; 2+, wheal from 7 to 12 mm.; 3+, wheal from 13 to 20 mm.; 4+, wheal over 20 mm.

Determination of the Effect of Migration of Allergen in Inciting Passive Transfer Reactions.—Twenty-four hours after sensitization, 0.025 ml. of CS-13 solution was injected intracutaneously into sites 1 and 3 for the CN and LC sera tests, respectively. The concentrations were adjusted so that the first recipient received 1 millimicrogram of CS-13 nitrogen and each subsequent recipient received a twofold increase in CS-13 nitrogen, the range being from 1 to 2,048 millimicrograms per test site. The size of the wheals in the injected and the uninjected sites was recorded thirty and sixty minutes after challenge.

TABLE II. NEUTRALIZATION OF REAGINS IN PASSIVELY SENSITIZED SITES BY MIGRATION OF ALLERGENS

RECIPIENT	SITE*	CS-13 NITROGEN (MγN)	REACTION IN 60 MINUTES†	
			LC SERUM	CN SERUM
J. H.	I	1	3+	3+
	U	0	4+	4+
H. M.	I	2	0	2+
	U	0	3+	4+
A. H.	I	4	0	0
	U	0	0	0
G. W.	I	8	0	0
	U	0	3+	4+
J. C.	I	16	0	0
	U	0	3+	3+
J. Ca.	I	32	0	0
	U	0	±	3+
J. M.	I	64	0	0
	U	0	3+	0
P. P.	I	128	0	0
	U	0	0	0
W. W.	I	256	0	0
	U	0	2+	0
J. D.	I	512	0	0
	U	0	0	0
R. B.	I	1,024	0	0
	U	0	0	0
H. T.	I	2,048	0	0
	U	0	0	0

*I refers to the site injected twenty-four hours earlier with the amount of CS-13 nitrogen shown under the next column to the right. U refers to the uninjected site. Results of this test were shown in Table I.

†Reaction sixty minutes after subcutaneous injection of 32 mcg. of CS-13 nitrogen into arm opposite that having the sensitized sites. Both sites I and U were negative when this challenge injection was made.

Control tests on the recipients by intracutaneous injection of 0.025 ml. of solution containing from 1 to 512 millimicrograms of CS-13 nitrogen were negative six days before the migration test. Results are shown in Table I.

Determination of the Effect of Migration of Allergen in Neutralizing Reagins in Passively Sensitized Sites.—Test for neutralization of reagins in both the injected and uninjected sites was made twenty-four hours after the intracutaneous challenge. One milliliter of solution containing 32 mcg. of CS-13

nitrogen was injected subcutaneously into the outer aspect of the upper arm opposite to that which had the sensitized sites. The size of the wheal was measured in both sites sixty minutes after challenge. Previous tests had shown that 32 mcg. of CS-13 nitrogen injected in this way was eight times greater than the threshold amount required to produce positive reactions in passively sensitized sites.[2] Control tests were not needed with this method of challenge.

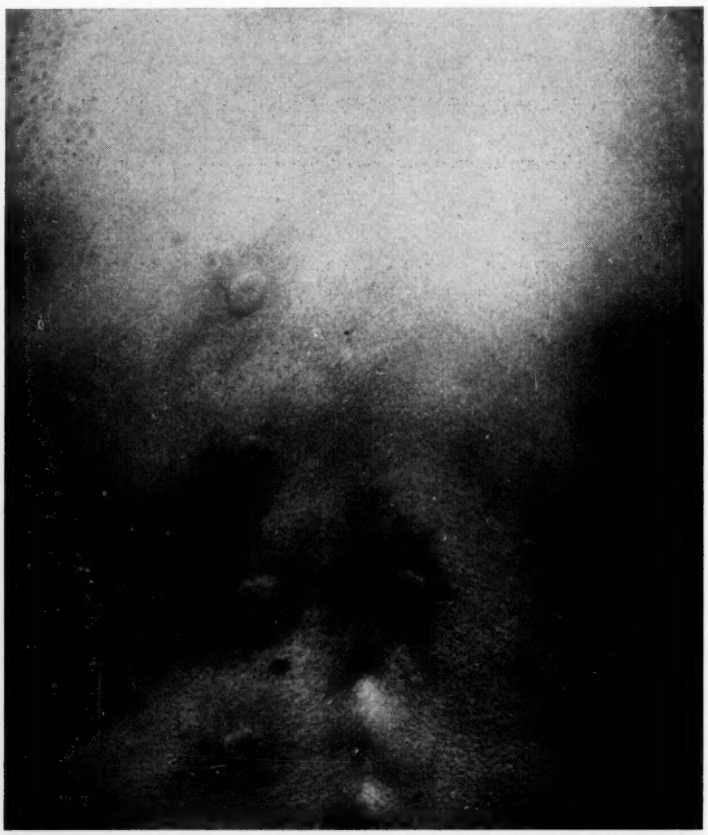

Fig. 1.—Photograph showing migration of CS-13 on back. Two mcg. of CS-13 nitrogen was injected intracutaneously into site designated with arrow thirty-two minutes before picture was taken. Surrounding, uninjected sites 50 mm. distant and 100 and 200 mm. above injected site reacted by migration of CS-13.

RESULTS AND DISCUSSION

The amount of CS-13 nitrogen required to incite positive passive transfer reactions and to neutralize reagins by migration of allergen from an injected site to an uninjected site 50 mm. distant was determined by testing with twofold serial dilutions over the range of 1 to 2,048 millimicrograms. Results are shown in Tables I and II. Reaction was incited in injected sites with both sera with all concentrations of allergen. The threshold amounts required to incite wheals by migration to uninjected sites were 1,024 and 2,048 millimicrograms of CS-13 nitrogen for the LC and CN sera, respectively. Redness, but no wheals, was

produced in a few uninjected sites with lesser amounts of CS-13 nitrogen. The threshold amounts of CS-13 nitrogen required to neutralize reagins by migration from the injected to the uninjected site, 50 mm. distant, were 512 and 64 millimicrograms with LC and CN sera, respectively. In contrast, neutralization of reagins in the injected sites was effected by 2 and 4 millimicrograms of CS-13 nitrogen with the LC and CN sera, respectively.

These results show that over 1,000 times as much allergen was required to incite reaction by migration to the uninjected site as was required to incite reaction by direct injection, but that only sixteen to 100 times as much allergen was required to neutralize the reagins by migration to the uninjected site as was required to neutralize reagins in the injected site. It is apparent that reagin neutralization can occur in uninjected sites by migration of allergen without any visible reaction.

The mechanism of migration of intracutaneously injected allergen to adjacent uninjected sites probably involves simple diffusion, as well as transport by lymph and blood circulatory systems. To determine which of these factors predominated, three sites, 50 mm. apart, were sensitized on the upper aspect of the biceps on the right arm and one site was sensitized on site 2, left forearm of a recipient, with 0.05 ml. CN serum injected in each site. Twenty-four hours after sensitization, the middle site of the group of three was injected intracutaneously with 0.025 ml. of solution containing 2,048 millimicrograms of CS-13 nitrogen. Sixty minutes after this challenge, wheals had formed as follows: right arm, lower site, 3+; middle (injected) site, 4+; upper site, 3+; site on left arm, negative. This indicated that migration for short distances involved diffusion primarily, for if circulatory transport were involved the site on the left arm also should have reacted. Circulatory transport undoubtedly predominates when allergen is absorbed. In another experiment, allergen was shown to migrate from a site on the biceps injected with 0.025 ml. of solution containing 1,024 millimicrograms of CS-13 nitrogen to give 3+ and 2+ reactions in sixty minutes, in sites 2 and 1, respectively, on the same forearm. This indicated that allergen migrated against the direction of lymph circulation. Fig. 1 shows a pattern of migration on the back, where movement of allergen was possibly more rapid than on the arms. Whereas uninjected sites were negative on the arms after thirty minutes, the sites on the back became red in fifteen minutes, with the wheals, as shown in Fig. 1, appearing in thirty minutes. The site marked with the arrow had been injected with 0.025 ml. of solution containing 2,048 millimicrograms of CS-13 nitrogen thirty-two minutes before the photograph was taken. The four sites surrounding the injected site are 50 mm. distant, while the sites above these are 100 and 200 mm. from the injected site. It is evident that migration occurred laterally as well as above and below the injected site.

The rate of migration of intracutaneously injected allergens probably varies, depending on their molecular weights. CS-13 may migrate faster than other allergens because the molecular weights of subfractions of CS-13 fractions ranged from 5,000 to 18,000.[2]

SUMMARY

Quantitative measurement of the migration of the cottonseed allergen, CS-13, in inciting positive passive transfer reactions and in neutralizing reagins by migration to an uninjected site 50 mm. from the injected site is described. Implications of these observations on interpretation of results obtained in comparing allergen fractions by multiple sensitized site testing on a single recipient are discussed. The results justify development of the method of evaluating allergens (described in our preceding article) which eliminates interference by migration.

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SEROTONIN, HISTAMINE, AND THE ARTHUS PHENOMENON

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THE Arthus phenomenon was first described in 1903.[1] Subsequently, numerous studies of this reaction have been made, but the factors causing it are not entirely clear. The phenomenon is best demonstrated in rabbits, although it can be produced in other species. In an adequately sensitized rabbit, the reaction is elicited by the intradermal injection of a small amount of the specific antigen. Shortly thereafter, erythema is seen at the site of the injection, followed by edema, further inflammation, and hemorrhage. In the more severe cases eventual ulceration, necrosis, and gangrene are found. The histologic changes of the lesion are typical.[2]

Histamine release has been implicated as one of the etiological factors in the Arthus reaction. The intradermal injection of histamine in large amounts failed to reproduce the Arthus lesion,[3] however, and antihistaminics have little or no effect on the reaction.[4]

Serotonin (5-hydroxytryptamine), as well as histamine, has been shown to be involved during anaphylaxis in rabbits.[5] In view of the lack of evidence to implicate histamine as one of the toxic factors in the production of the Arthus reaction, the possibility should be considered that the release of serotonin may be important.

Reserpine in large intravenous doses (5 mg. per kilogram) liberates most of the serotonin present in blood[6] and tissues[7] within sixteen to eighteen hours. At the same time, histamine is almost completely released from the blood[6] but not to a great extent from other tissues.[8] The serotonin and histamine content of the blood remains low for two to three days after the single injection of reserpine and then gradually returns to normal levels in about six days.[6] The present investigation was designed to study the Arthus reaction in rabbits treated with reserpine. If serotonin is important in the production of the phenomenon, the reaction should be inhibited in rabbits depleted of this amine.

METHODS

For this study, twenty rabbits were sensitized by the intraperitoneal injection of a saturated solution of crystalline egg albumin and used only after complement-fixation antibody titers were high. Reserpine (5 mg. per kilogram)

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was injected intravenously into ten of the rabbits; the other ten were kept as control animals. After sixteen hours, all the rabbits were given 0.1 ml. of the crystalline egg albumin solution intradermally. After two days, three of the rabbits that had received reserpine and had developed large hemorrhagic skin lesions were killed. The area of skin involved in the Arthus reaction was excised, and for control tissue an adjacent portion of normal skin was used. The lesions and the sections of normal skin were minced and homogenized individually in 0.1 N hydrochloric acid. Serotonin and histamine determinations were done on each homogenate as previously described.[9]

RESULTS

Reserpine produced marked sedation and diarrhea in the experimental rabbits during the first twenty-four hours after its administration. Erythema and edema developed at the site of injection of egg albumin in all the animals. In eight of the reserpine-treated rabbits and in seven of the control animals, the skin reaction progressed to form large hemorrhagic lesions. There was no apparent difference between the experimental and control rabbits in the rate at which the reaction developed or in the gross appearance of the lesions.

The analytical results (Table I) show that essentially no serotonin was present in the lesions or in the normal skin. The histamine concentration of the skin involved in the Arthus reaction was elevated and was much higher than the adjacent normal skin. The concentration of serotonin and of histamine was still below normal in blood drawn at the time the animals were killed.

TABLE I. SEROTONIN AND HISTAMINE CONCENTRATION IN SKIN AND BLOOD*

MATERIAL ANALYZED	SEROTONIN (γ/GM. OR ML.)	HISTAMINE (γ/GM. OR ML.)
Experimental skin	< 0.04†	6.3 (4.4-8.6)‡
Control skin	< 0.04†	1.0 (0.8-1.5)‡
Experimental blood	0.8	0.9
Normal range for whole blood	3.3-6.0	1.6-4.0

*Sixteen hours after intravenous reserpine (5 mg. per kilogram), rabbits sensitive to egg albumin were given intradermally 0.1 ml. of saturated crystalline egg albumin solution. Blood was drawn forty-eight hours later, and the rabbits were killed. The Arthus lesion and adjacent normal skin were homogenized in 0.1 N hydrochloric acid. The homogenates and the blood were analyzed for serotonin and histamine. The results were the average from three animals.

†The symbol < indicates that a reading was obtained on the spectrophotofluorometer but there was no distinct fluorescent peak for serotonin.

‡Range.

DISCUSSION

The Arthus reaction has been considered to be a direct consequence of the union of antigen with corresponding antibody. Studies by several investigators[10-12] have shown a definite relationship between the degree of sensitivity and the precipitin titer of the serum, suggesting that the Arthus phenomenon is dependent on the interaction of circulating precipitin and its specific antigen within the tissues. Beracerraf and Kabat[13] found that the presence of precipitating antibody was essential in production of the Arthus reaction. In addition to an adequate amount of antibody, antigen, and their combination, the

presence of a sufficient number of blood vessels is required in that tissue in which the reaction occurs.

Recently serotonin, as well as histamine, has been shown to be released into the blood plasma when antigen is injected intravenously into sensitized rabbits.[5] Subsequent study indicated that most of the serotonin was liberated from the circulating platelets.[14] It has been shown, both in vitro and in vivo, that antigen-antibody precipitate causes the release of both serotonin and histamine from the platelets of nonsensitized normal rabbits.[15] The relative platelet content of serotonin and histamine is much higher in the rabbit than in other animal species. During antigen-antibody reaction in sensitized rabbits, and following injection of the antigen-antibody complex into normal rabbits, clumping of the platelets and white blood cells occurs. These circulating platelet-leukocyte clumps are caught transiently in the lungs.[15] Glycogen, in vitro and in vivo, produces changes comparable to the antigen-antibody precipitate.[16]

Stetson[17] has demonstrated that the characteristic cutaneous lesions of the Arthus reaction are the result of the thrombosis of capillaries and small veins with clumps of platelets and leukocytes. These thrombi occlude the vessels and may be a factor in causing necrosis, hemorrhage, and tissue destruction. In addition, Stetson[18] has shown distinct similarities between the Arthus and Shwartzman phenomena and speculated that these reactions might be produced by the same mechanisms. The possible role of a polysaccharide in the Arthus reaction and during anaphylaxis should be investigated.

It would appear probable that, in addition to clumping of the platelets and leukocytes during the formation of the Arthus lesion, serotonin as well as histamine is released from the platelets. Since serotonin depletion in blood and tissues did not inhibit the formation of the Arthus reaction, it seems unlikely that serotonin is a factor in the production of this phenomenon. The finding that histamine or a histaminelike substance is elevated in the lesion itself again raises the question of any likely role for this amine in the Arthus reaction.

SUMMARY

Serotonin has been shown to be released during anaphylaxis in the rabbit. The possibility that this amine might be an etiological factor in the formation of the Arthus phenomenon was considered. Because the reaction develops readily in rabbits depleted of serotonin by reserpine, it appears doubtful that this amine is of importance in the production of the lesion.

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AMERICAN ACADEMY OF ALLERGY

PRESIDENTIAL ADDRESS

Francis C. Lowell, M.D., Boston, Mass.

THIS tropical environment is not at all conducive to a serious presidential address. This should not raise your expectations, however, for my subject probably weighs upon many of you as it certainly does upon me and it will lead me to tread a path well beaten by others in our field. Accordingly, prepare yourselves for that which is neither entertaining nor novel and take courage in the assurance that I will be brief.

Attention has been called repeatedly to the sorry plight of allergy and allergists in many teaching institutions; one aspect of this is the reluctance of the heads of many departments of medicine and the deans of many medical schools to make as much room for us in the curriculum as the broad and fascinating field of allergy would seem to warrant. As far as I am aware, the remedies suggested have done little to improve the situation. I am not aware, however, that anyone has seriously considered (in public) *why* we are used so poorly. It is to this prickly subject and with no little trepidation that I plan to address myself in the coming minutes.

To facilitate my task, I shall restrict my discussion to a single aspect of our professional activities, namely, the treatment of pollinosis with injections of allergenic extracts. My reason for selecting this particular aspect is that it is with injection therapy, as I shall call it, that we as allergists are chiefly identified. To the American public, an allergist is a doctor who gives shots. Indeed, this is so generally familiar that not long ago the premature headline announcements of an inadequately tested modification of injection therapy was meaningfully but misleadingly described by putting the term "shots" in the singular. Have you ever considered the degree of public familiarity with injection therapy required to make this possible? Now, is the fact of this identification good for our reputation among our professional colleagues? The answer is going to depend on how well we have laid the foundations for this form of treatment, how well we can answer questions about it, and the extent to which we have established the indications. In other words, it will depend on how well we have met our responsibility toward the patients we treat, the students we teach, and the ideals we live by. It seems to me that it is precisely here that our trouble lies, for the rationale for injection therapy is based chiefly on concepts borrowed

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from immunology and on impressions that have not yet been validated by experiments characterized by that type of rigid control which will reasonably exclude a role for chance or bias.

What would happen to the patient with pollinosis over the years if he were to forego injection therapy entirely? We cannot answer this, for it is rarely indeed that injection therapy is withheld from a patient with the "pollinosis syndrome" in clear form. Most of those we treat do well, to be sure, and our patients are certainly grateful. Nevertheless, we cannot rule out the possibility of a happy coincidence—the initiation of treatment in a year of less intense exposure to pollen, instruction in allergic cleanliness and in the intelligent use of drugs, the allaying of anxiety, spontaneous lessening of the patient's level of clinical sensitivity, and, last but by no means the least, the suggestion that accompanies the ritual of injection therapy. These, individually or in combination, might explain many a success without invoking any specific therapeutic or prophylactic merit for the solution that we put into the syringe.

And how many failures are explained away with the statement that "this was an exceptionally bad season" or that an inadequate dose was given, or by the acquisition of a cat, by hostility toward the allergist (Heaven forbid!), or by a neurotic reluctance to part with a chronic disease? In this subjective tangle, no casual observation can hope to appraise the role played by the solution injected. I am aware, of course, that several studies have been done with results favorable to injection therapy, and this is certainly fortunate, but I doubt that any study that has been done so far will withstand the scrutiny of that expert in demolition, the biostatistician.

I feel that we must admit that the value of injection therapy is far from settled. I think that we acknowledge this tacitly when we maintain an open mind about the value of injection schedules among which the top dosage advocated may vary by a factor of 1,000. To be sure, to keep a mind open is a good thing. However, rather than being a goal in itself, open mindedness is a state of expectancy in which one awaits the information that warrants closing the mind, taking care of course not to slam it shut on insufficient evidence and thus fall into the error of prejudice. A little more of this, and I will have finished moralizing. Beware the comfort to be derived from the attitude of the open mind. When we say that "there may be something in it," we may be judiciously postponing an opinion for lack of evidence. But is it not conceivable that the feeling of unassailability which so tolerant an attitude gives may soothe our anxieties to the point where we make no effort to obtain the evidence? Whether this is true or not, you will have long since guessed that my address today constitutes a plea that the Academy as an organization and we as members of it apply ourselves to the problem of assessing the value of injection therapy.

There is little point in urging the impossible, and among the objections that one might raise is the fact that the variables are so numerous that no answer could come from any study of manageable size. If this objection is valid, not only would further study of the efficacy of injection therapy be futile, but we

would also have to acknowledge that it is on the weakest of hunches that we urge or even permit our patients to go to the expense and trouble of receiving injection therapy. On the other hand, if injection therapy warrants the expense and trouble, then its efficacy should become apparent in a suitably designed study. Successful studies characterized by rigid experimental control have been carried out in other areas of clinical medicine in which the problem of experimental design would appear to have been equally difficult, and it is my opinion that similar techniques can be successfully applied to the evaluation of injection therapy. This will be no easy job. Since we probably have much to learn before useful data will be forthcoming, I would urge that this be considered a continuing effort over the coming years. And, since a successful study will require many patients and concerted effort, it is my belief that the number of injection schedules studied should be held to a minimum.

In closing, I would like to add that such an effort will certainly have far-reaching effects, all of which seem to me desirable. We will acquire the art (and I use the word advisedly) of conducting sound experiments which we can then apply in other areas. We will learn much that we do not yet know concerning the natural history of pollinosis, we will sharpen our critical faculties, we will acquire greater sophistication in the assessment of data culled in a complex clinical setting, and perhaps as a consequence of all this—and I now return to the beginning—we will be better used.

ANNOUNCEMENTS

Southwest Allergy Forum

The Southwest Allergy Forum will be held at the Skirvin Hotel in Oklahoma City, Oklahoma, April 2 to 5, 1960. A very interesting program is planned, and all allergists in this area are invited.

George L. Winn, M.D.
Secretary, Southwest Allergy Forum
711 Northwest Tenth
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The American Association for the Study of Headache

Recently a new society was formed which is to be known as the American Association for the Study of Headache. The purpose behind this organization is to bring together men who are interested in the study of headache, and to allow them to discuss and express their viewpoints on current trends in this field. At the present time it is planned to have one formal meeting before the annual meeting of the American Medical Association. An informal meeting was held during the Southern Medical Association meeting in Atlanta. Officers include: *President,* Henry D. Ogden, M.D., 560 S. Galvez, New Orleans, La.; *Vice-President,* Walter C. Alvarez, M.D., Chicago, Ill.; *Treasurer,* Robert E. Ryan, M.D., St. Louis, Mo.; *Secretary,* Bayard T. Horton, M.D., Rochester, Minn.; Martin Tunis, M.D., Moderator, Montreal, Quebec, Canada.

BOOK REVIEWS

"Allergic" Encephalomyelitis. M. W. Kies and E. C. Alvord, Jr. (editors). Springfield, Ill., 1959, Charles C Thomas, Publisher. 576 pages.

This book is a report of the proceedings of a symposium on experimental "allergic" encephalomyelitis and its relation to other diseases of man and animals, in which sixty investigators, prominent in the fields of pathology, neurology, immunology, and biochemistry, participated. The major portion of the book consists of twelve prepared addresses, interspersed liberally with discussions of these papers by the participants; these have been recorded verbatim and edited for readability.

The vast amount of material covered in the book is presented in three sections, each dealing with a major area of investigation in this field. The first section, called "Clinico-Pathologic Correlations," consists of papers bearing on the histologic and pathologic nature of the experimental form of encephalomyelitis, of the rabies postvaccinal encephalomyelitis in man, and of other human demyelinating diseases. These papers, liberally illustrated with photographs of histologic sections, seek to describe and correlate the experimentally produced lesions with certain human diseases of as yet unknown etiology (multiple sclerosis, for example). That much disagreement exists among the experts as to the basic nature of the observed lesions is indicated by the very spirited discussion section following these papers. Unfortunately, it must be stated that a good part of this is concerned with that ever-present problem in histology, the nomenclature of cells, a subject with which the novice tends to become impatient.

The second section of the book is concerned with work on the chemical nature of the antigen or antigens involved in the production of experimental encephalomyelitis and contains much technical information on the fractionation, purification, and characterization of active lipids and water-soluble materials from nervous tissue. While the problem of assay of encephalitogenic activity is one of general interest to immunology, the chemical studies will probably appeal only to those closely involved, since the techniques used are not such as to be generally applicable.

The last section, on immunologic aspects of the disease, should prove of greatest interest to allergists and immunologists. Here are included much excellent material and discussion on a still unsolved question fundamental to our understanding of all auto-allergic disease, that is, the relative importance and role of circulating antibody and the cellular type of hypersensitivity, a question which gives rise to much excellent, thought-provoking discussion.

The avowed purpose of this symposium was to assemble all available data on "allergic" encephalomyelitis, to examine critically whether this condition was indeed due to an allergic reaction, and finally to evaluate the relation among these various disorders. In this, the contributors have succeeded excellently, and this book, by combining in one readily accessible source a wealth of material, should be an invaluable aid to people working in this area. In another, more general, sense this book should be of great interest to immunologists. By focusing in a critical and comprehensive fashion on probably the most studied of the "auto-allergic" diseases, this book will undoubtedly provide a stimulus to renewed efforts on some of the poorly understood diseases considered by some to have an immunologic etiology, such as the so-called "collagen" group. One can only hope that these new efforts will be as intelligently and critically pursued as were the contributions in this book.

Sidney Leskowitz

Praktikum der allergischen Krankheiten. G. A. Rost, D. G. R. Findeisen, and I. Niemand-Anderssen. Leipzig, 1958, Johann Ambrosius Barth. 275 pages, 37 illustrations.

Der Schnupfen Beiträge zur Ätiologie, Pathogenese, Diagnostik und Therapie mit besonderer Berücksichtigung der Allergie. G. Eigler and D. G. R. Findeisen (editors). Leipzig, 1959, Johann Ambrosius Barth. 132 pages, 20 illustrations.

In spite of reservations, two new German books on allergy illustrate the impressive growth and vitality of the specialty on the Continent.

The *Manual of Allergic Diseases* (the "Praktikum") shows clearly that the attempt to present the theoretical concepts of allergy in a small monograph is doomed to failure. Rost resorts to dogma which as times distorts the existing evidence. On page 2, for instance, he presents a startling bit of misinformation which hints that the incidence of allergic diseases in the American Negro is approximately the same as the incidence in the non-Negro population. A review of the reference upon which this statement is based reveals that it refers to Chinese (not to Negroes) and that it limits its survey to lichen simplex chronicus, which it considers a psychogenic rather than an allergic disease.

Many of the basic chapters (for example, the chapter on infection and allergy), omit significant work of recent origin. The chapter on infections, by the way, ends with a rather peculiar paragraph on "salt allergy" which lacks conviction.

The practical chapters are good. The sections on diagnosis are adequate, although there are, of course, areas of disagreement. The endorsement of the leukopenic index, to name an example, is strong but vague and disregards the studies of its statistical validity which have been carried out in the United States.

The approach to treatment is conservative, with justified emphasis on the preventive aspects of allergic disease. It might be mentioned, on the other hand, that the chapter on antihistamine therapy will be of little use to non-European readers; it consists essentially of a long tabulation of names, dosages, and manufacturers of antihistamine drugs made in Germany.

Unlike the "Prakticum," the *Symposium on "Coryza"* presents an excellent summary of physiology and pathology of the upper respiratory tract. The fundamental discussion accounts for more than one-half of the monograph; the remainder discusses clinical management.

Again, the book represents a middle-of-the-road approach. This is surely to the credit of Findeisen, who must have been the guiding spirit for both of these volumes. At times the gap between theory and practice becomes apparent; Hajos' so-called specific desensitization with autogenous vaccines and with extracts of polypoid tissue might be successful, but its theoretical foundation is open to debate. Topical medication is competently described in a special chapter (Naumann).

Surgical results remain equivocal. Majer's finding that nasal polyps are distinguished by rapid proliferation of polygonal, silver-positive, cells with reticular fibers is interesting; his conclusion that removal of these granulomatous structures will remove a receptor system and, therefore, "like the removal of a focus of infection" will break up the vicious cycle between nasal polyps and bronchial asthma seems perhaps more optimistic than warranted by clinical experience.

The final chapter of the volume deals with the occupational significance of nasal reactions (Dishoeck).

It is gratifying to recognize that this monograph is a result of close cooperation between internists and otolaryngologists and that European otolaryngology has developed a strong interest in the structure and function of the nasal mucous membrane. The book, although smaller in scope, stands comparison with Hansel's classical work on the nose and the paranasal sinuses; this, indeed, is no mean compliment.

M. S.